

We claim:

1. A method for obtaining a broad spectrum antibody against an antigen of interest, comprising:

(a) priming a non-human animal by administering a first immunogen derived from the antigen of interest, wherein the non-human animal comprises:

(i) a genetically modified immunoglobulin heavy chain locus comprising one or more human V_H gene segments, a plurality of human D_H gene segments, and a plurality of human J segments, wherein the V_H , D_H , and J_H gene segments are operably linked to a heavy chain constant region nucleic acid sequence,

(ii) a genetically modified immunoglobulin light chain locus comprising one or more human V_K gene segments and one or more human J_K gene segments, wherein the V_K and the J_K gene segments are operably linked to a light chain constant region nucleic acid sequence, and

wherein the first immunogen comprises a plurality of epitopes;

(b) allowing the non-human animal to mount an immune response against the first immunogen, wherein the immune response comprises generation of non-human B cells that express human immunoglobulin heavy chain (IgH) VDJ and human immunoglobulin light chain (IgL) VJ sequences;

(c) isolating clonally related B cells from the non-human animal that express a B cell receptor (BCR) that specifically binds a first epitope of the first immunogen;

(d) obtaining IgH VDJ and IgL VJ amino acid sequences expressed by the B cell receptor (BCR) of the clonally related B cells;

(e) deducing from the IgH VDJ and IgL VJ sequences unmutated B cell receptor (BCR) VDJ and VJ amino acid sequences; and one or more intermediate ancestor B cell receptor (BCR) VDJ and VJ amino acid sequences expressed by B cells at an intermediate stage of differentiation;

(f) designing a plurality of immunogens that bind with enhanced affinity to the unmutated B cell receptor (BCR) or the intermediate ancestor B cell receptor (BCR);

(g) boosting the immune response in the non-human animal by administering to the non-human animal a second immunogen selected from the plurality of the immunogens in (f), wherein the second immunogen comprises a second epitope distinct from the first epitope; and

(h) obtaining the broad spectrum antibody from the non-human animal of (g).

2. The method of claim 1, wherein the immune response of the non-human animal is boosted by serially administering the plurality of immunogens identified in step (e) until the broad spectrum antibody is produced by the non-human animal.

3. The method of claim 1, wherein the immune response of the non-human animal is boosted by administering a combination of the plurality of the immunogens identified in step (e).

4. The method of claim 1, wherein the first and the second immunogens are administered as a liposomal complex.

5. The method of claim 1, wherein the antigen of interest is, or is derived from, an infectious agent or a pathogen, and the broad spectrum antibody is a broadly neutralizing antibody against the infectious agent or the pathogen.

6. The method of claim 5, wherein the infectious agent or pathogen is selected from the group consisting of a virus, a bacterium, a fungus, and a parasite.

7. The method of claim 5, wherein the infectious agent is selected from the group consisting of a human immunodeficiency virus (HIV), a hepatitis A virus, a hepatitis B virus, a hepatitis C virus, a dengue fever virus, and a human papillomavirus (HPV).

8. The method of claim 1, wherein all, or substantially all, functional endogenous V_H , D_H , and J_H gene segments in the immunoglobulin heavy chain locus have been deleted or rendered non-functional, and wherein the genetically modified immunoglobulin heavy chain locus comprises a single human V_H gene segment or a polymorphic variant thereof, one or more human D_H gene segment, and one or more human J_H gene segment.

9. The method of claim 8, wherein the genetically modified heavy chain locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification in the heavy chain locus does not affect the expression or function of the endogenous Adam6a gene, Adam6b gene, or both.

10. The method of claim 8, wherein the non-human animal comprises an ectopically present Adam6a gene, Adam6b gene, or both.
11. The method of claim 8, wherein the single human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81 gene segment, and a polymorphic variant thereof.
12. The method of claim 8, wherein the single human V_H gene segment is a human V_H1-69 gene segment or a polymorphic variant thereof.
13. The method of claim 8, wherein the single human V_H gene segment is a human V_H1-2 gene segments or a polymorphic variant thereof.
14. The method of claim 8, wherein the single human V_H gene segment is a human V_H4-59 gene segment or a polymorphic variant thereof.
15. The method of claim 1, wherein the non-human animal is a rodent.
16. The method of claim 1, wherein the non-human animal is a mouse or a rat.
17. The method of claim 1, wherein the constant region nucleic acid sequence is a human or a rodent nucleic acid sequence.
18. The method of claim 1, wherein the non-human animal is a mouse and the constant region nucleic acid sequence is a mouse constant region nucleic acid sequence.
19. The method of claim 1, wherein all, or substantially all, functional endogenous V_H, D_H, and J_H gene segments in the immunoglobulin heavy chain locus have been rendered non-functional, and the human V_H, D_H, and J_H gene segments are present on a transgene.
20. The method of claim 1, where the human V_H, D_H, and J_H gene segments are operably linked to an endogenous constant region nucleic acid sequence at an endogenous non-human immunoglobulin heavy chain locus.

21. The method of claim 1, wherein the genetically modified immunoglobulin light chain locus comprises a replacement of all, or substantially all, functional V_κ gene segments and J_κ gene segments with one or more human V_κ gene segments and one or more J_κ gene segments, wherein the human V_κ gene segments and the J_κ gene segments are operably linked to a light chain constant region nucleic acid sequence.

22. The method of claim 1, wherein the non-human animal comprises a replacement at the endogenous non-human immunoglobulin V_κ locus of all or substantially all functional endogenous V_κ gene segments with human V_κ gene segments selected from V_κ4-1, V_κ5-2, V_κ7-3, V_κ2-4, V_κ1-5, V_κ1-6, V_κ3-7, V_κ1-8, V_κ1-9, V_κ2-10, V_κ3-11, V_κ1-12, V_κ1-13, V_κ2-14, V_κ3-15, V_κ1-16, V_κ1-17, V_κ2-18, V_κ2-19, V_κ3-20, V_κ6-21, V_κ1-22, V_κ1-23, V_κ2-24, V_κ3-25, V_κ2-26, V_κ1-27, V_κ2-28, V_κ2-29, V_κ2-30, V_κ3-31, V_κ1-32, V_κ1-33, V_κ3-34, V_κ1-35, V_κ2-36, V_κ1-37, V_κ2-38, V_κ1-39, V_κ2-40, and a combination thereof.

23. The method of claim 1, wherein the non-human animal comprises a replacement at the endogenous non-human immunoglobulin J_κ locus of all or substantially all functional endogenous non-human immunoglobulin J_κ gene segments with human J_κ gene segments selected from J_κ1, J_κ2, J_κ3, J_κ4, J_κ5, and a combination thereof.

24. The method of claim 21, wherein the one or more human V_κ and J_κ segments are operably linked to an endogenous light chain constant region nucleic acid sequence at an endogenous non-human locus.

25. A broad spectrum antibody produced by the method of claim 1.

26. A broad spectrum antibody produced by the method of claim 2.

27. A broad spectrum antibody produced by the method of claim 3.

28. A broad spectrum antibody produced by the method of claim 4.

29. A broadly neutralizing antibody against an infectious agent or a pathogen produced by the method of claim 4.

CLAIMS

1. A method for obtaining a broad spectrum antibody against an antigen of interest, comprising:

(a) priming a non-human animal by administering a first immunogen derived from the antigen of interest, wherein the non-human animal comprises:

- i. a genetically modified immunoglobulin heavy chain locus comprising one or more human V_H gene segments, a plurality of human D_H gene segments, and a plurality of human J segments, wherein the V_H , D_H , and J_H gene segments are operably linked to a heavy chain constant region nucleic acid sequence,
- ii. a genetically modified immunoglobulin light chain locus comprising one or more human V_K gene segments and one or more human J_K gene segments, wherein the V_K and the J_K gene segments are operably linked to a light chain constant region nucleic acid sequence, and wherein the first immunogen comprises a plurality of epitopes;

(b) allowing the non-human animal to mount an immune response against the first immunogen, wherein the immune response comprises generation of non-human B cells that express human immunoglobulin heavy chain (IgH) VDJ and human immunoglobulin light chain (IgL) VJ sequences;

(c) isolating clonally related B cells from the non-human animal that express a B cell receptor (BCR) that specifically binds a first epitope of the first immunogen;

(d) obtaining IgH VDJ and IgL VJ amino acid sequences expressed by the B cell receptor (BCR) of the clonally related B cells;

(e) deducing from the IgH VDJ and IgL VJ sequences unmutated B cell receptor (BCR) VDJ and VJ amino acid sequences; and one or more intermediate ancestor B cell receptor (BCR) VDJ and VJ amino acid sequences expressed by B cells at an intermediate stage of differentiation;

(f) designing a plurality of immunogens that bind with enhanced affinity to the unmutated B cell receptor (BCR) or the intermediate ancestor B cell receptor (BCR);

(g) boosting the immune response in the non-human animal by administering to the non-human animal a second immunogen selected from the plurality of the immunogens

in (f), wherein the second immunogen comprises a second epitope distinct from the first epitope; and

(h) obtaining the broad spectrum antibody from the non-human animal of (g).

2. The method of claim 1, wherein

(a) the immune response of the non-human animal is boosted by serially administering the plurality of immunogens identified in step (e) until the broad spectrum antibody is produced by the non-human animal; or

(b) the immune response of the non-human animal is boosted by administering a combination of the plurality of the immunogens identified in step (e).

3. The method of any one of claims 1-2, wherein the first and the second immunogens are administered as a liposomal complex.

4. The method of any one of claims 1-3, wherein the antigen of interest is, or is derived from, an infectious agent or a pathogen, and the broad spectrum antibody is a broadly neutralizing antibody against the infectious agent or the pathogen.

5. The method of claim 4, wherein the infectious agent or pathogen is selected from the group consisting of a virus, a bacterium, a fungus, and a parasite.

6. The method of claim 4, wherein the infectious agent is selected from the group consisting of a human immunodeficiency virus (HIV), a hepatitis A virus, a hepatitis B virus, a hepatitis C virus, a dengue fever virus, and a human papillomavirus (HPV).

7. The method of any one of claims 1-6, wherein all, or substantially all, functional endogenous V_H , D_H , and J_H gene segments in the immunoglobulin heavy chain locus have been deleted or rendered non-functional, and wherein the genetically modified immunoglobulin heavy chain locus comprises a single human V_H gene segment or a polymorphic variant thereof, one or more human D_H gene segment, and one or more human J_H gene segment.

8. The method of claim 7, wherein

(a) the genetically modified heavy chain locus comprises an endogenous Adam6a gene, Adam6b gene, or both, and the genetic modification in the heavy chain locus does not affect the expression or function of the endogenous Adam6a gene, Adam6b gene, or both; or

(b) the non-human animal comprises an ectopically present Adam6a gene, Adam6b gene, or both.

9. The method of claim 7, wherein the single human V_H gene segment is selected from the group consisting of V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81 gene segment, and a polymorphic variant thereof.

10. The method of claim 7, wherein

(a) the single human V_H gene segment is a human V_H1-69 gene segment or a polymorphic variant thereof;

(b) the single human V_H gene segment is a human V_H1-2 gene segments or a polymorphic variant thereof; or

(c) the single human V_H gene segment is a human V_H4-59 gene segment or a polymorphic variant thereof.

11. The method of any one of claims 1-10, wherein the non-human animal is a rodent.

12. The method of any one of claims 1-10, wherein the non-human animal is a mouse or a rat.

13. The method of any one of claims 1-10, wherein the constant region nucleic acid sequence is a human or a rodent nucleic acid sequence.

14. The method of any one of claims 1-10, wherein the non-human animal is a mouse and the constant region nucleic acid sequence is a mouse constant region nucleic acid sequence.

15. The method of any one of claims claim 1-14, where the human V_H, D_H, and J_H gene segments are operably linked to an endogenous constant region nucleic acid sequence at an endogenous non-human immunoglobulin heavy chain locus.

16. The method of any one of claims 1-15, wherein the genetically modified immunoglobulin light chain locus comprises

(a) a replacement of all, or substantially all, functional V_κ gene segments and J_κ gene segments with one or more human V_κ gene segments and one or more J_κ gene

segments, wherein the human V κ gene segments and the J κ gene segments are operably linked to a light chain constant region nucleic acid sequence; and/or

(b) a replacement at the endogenous non-human immunoglobulin V κ locus of all or substantially all functional endogenous V κ gene segments with human V κ gene segments selected from V κ 4-1, V κ 5-2, V κ 7-3, V κ 2-4, V κ 1-5, V κ 1-6, V κ 3-7, V κ 1-8, V κ 1-9, V κ 2-10, V κ 3-11, V κ 1-12, V κ 1-13, V κ 2-14, V κ 3-15, V κ 1-16, V κ 1-17, V κ 2-18, V κ 2-19, V κ 3-20, V κ 6-21, V κ 1-22, V κ 1-23, V κ 2-24, V κ 3-25, V κ 2-26, V κ 1-27, V κ 2-28, V κ 2-29, V κ 2-30, V κ 3-31, V κ 1-32, V κ 1-33, V κ 3-34, V κ 1-35, V κ 2-36, V κ 1-37, V κ 2-38, V κ 1-39, V κ 2-40, and a combination thereof; and/or

(c) a replacement at the endogenous non-human immunoglobulin J κ locus of all or substantially all functional endogenous non-human immunoglobulin J κ gene segments with human J κ gene segments selected from J κ 1, J κ 2, J κ 3, J κ 4, J κ 5, and a combination thereof.

17. The method of claim 16, wherein the one or more human V κ and J κ segments are operably linked to an endogenous light chain constant region nucleic acid sequence at an endogenous non-human locus.

18. A broad spectrum antibody produced by the method of any one of claims 1-17.