

CLAIMS

What is claimed is:

1. A genetically modified non-human rodent whose germline genome comprises:
 - (a) a nucleotide sequence encoding a human or humanized MHC molecule that comprises at least a human peptide-binding groove of a human leukocyte antigen (HLA) molecule or at least the human peptide-binding groove thereof, and
 - (b) one or more immunoglobulin (Ig) loci selected from the group consisting of:
 - (i) an unrearranged or rearranged human or humanized Ig heavy chain locus;
 - (ii) an unrearranged or rearranged human or humanized Ig light chain locus; or
 - (iii) an unrearranged or rearranged human or humanized Ig heavy chain locus, and an unrearranged or rearranged human or humanized Ig light chain locus,wherein the genetically modified rodent expresses the human or humanized MHC molecule or at least the human peptide-binding groove thereof,
wherein the genetically modified rodent expresses in a B cell a human or humanized antibody comprising:
 - (A) a human or humanized heavy chain variable domain;
 - (B) a human or humanized light chain variable domain; or
 - (C) a human or humanized heavy chain variable domain and a human or humanized light chain variable domain, andwherein the human or humanized antibody binds an antigenic peptide-MHC (pMHC) that comprises:
 - (I) a peptide that is heterologous to the genetically modified rodent complexed with
 - (II) the HLA molecule or at least the human peptide-binding groove thereof.
2. The genetically modified rodent of claim 1, wherein the human or humanized heavy chain variable domain is expressed from a rearranged Ig heavy chain variable region locus, wherein said rearranged Ig heavy chain variable region locus is formed from said unrearranged human or humanized Ig heavy chain locus.

3. The genetically modified rodent of claim 1, wherein the human or humanized light chain variable domain is expressed from a rearranged Ig light chain variable region locus, wherein said rearranged Ig light chain variable region locus is formed from said unrearranged human or humanized Ig light chain locus.
4. The genetically modified rodent of any one of claims 1-3, wherein the human or humanized antibody comprises an IgG isotype.
5. The genetically modified rodent of any one of claims 1-4, wherein the human or humanized MHC molecule is selected from the group consisting of a human or humanized MHC class I molecule, a human or humanized MHC class II α molecule, a human or humanized MHC class II β molecule, or any combination thereof.
6. The genetically modified rodent of any one of claims 1-5, wherein the genetically modified rodent comprises:
 - (a) at an endogenous Ig heavy chain locus:
 - (i) an unrearranged human or humanized Ig heavy chain variable region in operable linkage to an endogenous Ig heavy chain constant region;
 - (ii) a restricted unrearranged human or humanized Ig heavy chain variable region in operable linkage to an endogenous Ig heavy chain constant region;
 - (iii) a common Ig heavy chain encoding sequence;
 - (iv) a histidine modified unrearranged human or humanized Ig heavy chain variable region in operable linkage to an endogenous Ig heavy chain constant region;
 - (v) a heavy chain only Ig encoding sequence; or
 - (vi) an unrearranged human or humanized hybrid Ig heavy chain sequence encoding a hybrid Ig chain;
 - and/or
 - (b) at an endogenous Ig light chain locus:
 - (i) an unrearranged human or humanized Ig light chain variable region in operable linkage to an endogenous Ig light chain constant region;
 - (ii) a common Ig light chain encoding sequence;

- (iii) a restricted unrearranged human or humanized Ig light chain variable region in operable linkage to an endogenous Ig light chain constant region;
 - (iv) a histidine modified unrearranged human or humanized Ig light chain variable region in operable linkage to an endogenous Ig light chain constant region; or
 - (v) a histidine modified rearranged human or humanized Ig light chain variable region in operable linkage to an endogenous Ig light chain constant region.
- 7. The genetically modified rodent of any one of claims 1-6, wherein the genetically modified rodent:
 - (i) further comprises a functional ADAM6 gene.
- 8. The genetically modified rodent of claim 7, wherein the functional ADAM6 gene is an endogenous ADAM6 gene.
- 9. The genetically modified rodent of any one of claims 1-8, wherein the genetically modified rodent further expresses an exogenous terminal deoxynucleotidyl transferase (TdT) gene.
- 10. The genetically modified rodent of any one of claims 1-9, wherein the human or humanized MHC molecule is a human or humanized MHC class I molecule.
- 11. The genetically modified rodent of claim 10, wherein the human peptide-binding groove is derived from an HLA class I molecule selected from the group consisting of an HLA-A molecule, an HLA-B molecule, and an HLA-C molecule.
- 12. The genetically modified rodent of claim 11, wherein the genome of the genetically modified rodent further comprises a nucleotide sequence encoding a human or humanized β 2 microglobulin.
- 13. The genetically modified rodent of claim 12, wherein the nucleotide sequence encoding the human or humanized β 2 microglobulin is at an endogenous β 2 microglobulin locus.

14. The genetically modified rodent of claim 12 or claim 13, wherein the genetically modified rodent expresses the human or humanized $\beta 2$ microglobulin such that the genetically modified rodent is tolerized to the human or humanized $\beta 2$ microglobulin by itself or in association with the human or humanized MHC class I molecule.
15. The genetically modified rodent of any one of claims 1-9, wherein the human or humanized MHC molecule is a human or humanized MHC class II molecule.
16. The genetically modified rodent of claim 15, wherein the human peptide-binding groove is derived from an α chain, a β chain, or an α chain and a β chain of an HLA class II molecule selected from the group consisting of an HLA-DP molecule, an HLA-DQ molecule, and an HLA-DR molecule.
17. The genetically modified rodent of any one of claims 1-16, wherein the nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof encodes a fully human MHC molecule.
18. The genetically modified rodent of claim 17, wherein the nucleotide sequence encoding the fully human MHC molecule does not disrupt an endogenous MHC locus.
19. The genetically modified rodent of claim 18, wherein the nucleotide sequence encoding the fully human MHC molecule is placed into an endogenous ROSA26 locus.
20. The genetically modified rodent of any one of claims 1-19, wherein the nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof encodes a chimeric human/rodent MHC molecule comprising extracellular domains of an HLA molecule operably linked to transmembrane and cytoplasmic domains of an endogenous MHC molecule.

21. The genetically modified rodent of claim 20, wherein the nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof encodes:

(i) a chimeric human/rodent MHC class I molecule comprising an $\alpha 1$ domain, an $\alpha 2$ domain, and an $\alpha 3$ domain of an HLA class I molecule selected from the group consisting of HLA-A, HLA-B, and HLA-C operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class I molecule, such as an endogenous murine H-2K molecule, an endogenous murine H-2D molecule, or an endogenous murine H-2L molecule, and/or

(ii) a chimeric human/rodent MHC class II molecule comprising an $\alpha 1$ domain and an $\alpha 2$ domain of an HLA class II α molecule operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II α molecule, such as an endogenous murine H-2A α molecule or an endogenous murine H-2E α molecule, and/or a $\beta 1$ domain and a $\beta 2$ domain of an HLA class II β molecule operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II β molecule, such as an endogenous murine H-2A β molecule or endogenous murine H-2E β molecule.

22. The genetically modified rodent of any one of claims 1-21, further comprising the antigenic pMHC that comprises (I) the peptide that is heterologous to the genetically modified rodent associated with (II) the HLA molecule or at least the human peptide-binding groove thereof.

23. The genetically modified rodent of any one of claims 1-22, wherein the human or humanized antibody that binds the antigenic pMHC does not bind an empty HLA molecule from which the human peptide-binding groove is derived.

24. The genetically modified rodent of any one of claims 1-23, wherein the genetically modified rodent is heterozygous for the nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof.

25. The genetically modified rodent of any one of claims 1-24, wherein:

- (i) the human or humanized Ig heavy chain locus is rearranged and the human or humanized Ig light chain locus is unrearranged, or
- (ii) the human or humanized Ig heavy chain locus is unrearranged and the human or humanized Ig light chain locus is rearranged.

26. The genetically modified rodent of any one of claims 1-25, wherein the genetically modified rodent is a rat or a mouse.

27. The genetically modified rodent of any one of claims 1-26, wherein the genetically modified rodent is a mouse.

28. A method of making the genetically modified rodent of any one of claims 1-27, comprising modifying the genome of a rodent to comprise:

- (a) the nucleotide sequence encoding the human or humanized MHC molecule that comprises at least a human peptide-binding groove of a human leukocyte antigen (HLA) molecule or at least the human peptide-binding groove thereof, and
- (b) one or more immunoglobulin (Ig) loci selected from the group consisting of:
 - (i) an unrearranged or rearranged human or humanized Ig heavy chain locus;
 - (ii) an unrearranged or rearranged human or humanized Ig light chain locus; or
 - (iii) an unrearranged or rearranged human or humanized Ig heavy chain locus and an unrearranged or rearranged human or humanized Ig light chain locus,

wherein the genetically modified rodent is:

(A) tolerized to the human or humanized MHC molecule or at least the human peptide binding groove thereof such that it generates a human or humanized antibody when immunized with an antigenic pMHC that comprises (i) a peptide that is heterologous to the genetically modified rodent complexed with (ii) the HLA molecule or at least the human peptide-binding groove thereof, and

(B) capable of expressing from a B-cell said human or humanized antibody comprising:

- (i) a human or humanized Ig heavy chain variable domain;
- (ii) a human or humanized Ig light chain variable domain; or

(iii) a human or humanized Ig heavy chain variable domain and a human or humanized Ig light chain variable domain.

29. The method of claim 28, wherein:

(i) the human or humanized Ig heavy chain locus is unrearranged;

(ii) the human or humanized Ig light chain locus is unrearranged; or

(iii) the human or humanized Ig heavy chain locus is unrearranged and the human or humanized Ig light chain locus is unrearranged.

30. The method of claim 28 or claim 29, wherein the method comprises

(a)

(1) inserting the nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof into a first ectopic locus, or

(2) replacing:

at an endogenous rodent MHC I locus, a nucleotide sequence encoding a rodent MHC I molecule with a nucleotide sequence encoding a chimeric human/rodent MHC I molecule, and/or

at an endogenous rodent MHC II locus, a nucleotide sequence encoding a rodent MHC II molecule with a nucleotide sequence encoding a chimeric human/rodent MHC II molecule, and

(b)

(i) inserting an unrearranged or rearranged human or humanized Ig heavy chain locus and/or an unrearranged or rearranged human or humanized Ig light chain locus into a second ectopic locus, or

(ii) replacing:

at an endogenous rodent Ig heavy chain locus, an endogenous rodent Ig heavy chain variable (V_H) gene segment with an unrearranged human V_H gene segment, and optionally replacing an endogenous rodent Ig heavy chain diversity (D_H) gene segment and/or an endogenous rodent Ig heavy chain joining (J_H) gene segment with an unrearranged human D_H gene segment and/or an unrearranged

human J_H gene segment, respectively, wherein the unrearranged human V_H, and optional unrearranged human D_H gene segment and optional unrearranged human J_H gene segment, are operably linked to an endogenous Ig heavy chain constant region gene sequence, and/or

at an endogenous rodent Ig light chain locus, an endogenous rodent Ig light chain variable (V_L) gene segment and an endogenous rodent Ig light chain joining (J_L) gene segment with a human V_L gene segment and a human J_L gene segment, which are optionally rearranged to form a V_L/J_L gene sequence, wherein the human V_L gene segment and the human J_L gene segment are operably linked to an endogenous Ig light chain constant region gene sequence,

wherein:

(A) the (a)(1) nucleotide sequence encoding the human or humanized MHC molecule or at least the human peptide-binding groove thereof and the (b)(i) unrearranged or rearranged human or humanized Ig heavy chain locus and/or unrearranged or rearranged human or humanized Ig light chain locus are:

(I) inserted by sequential homologous recombination in a single rodent embryonic stem (ES) cell; or

(II) inserted into a first ES cell and a second ES cell, wherein said first ES cell and second ES cell are used respectively to generate a first genetically modified rodent and a second genetically modified rodent; or

(B) the (a)(2) nucleotide sequence encoding the rodent MHC I molecule and/or nucleotide sequence encoding the rodent MHC II molecule, and (b)(ii) the endogenous rodent V_H, and optional D_H and/or J_H gene segments, and/or the V_L and J_L gene segments are:

(I) replaced by sequential homologous recombination in a single rodent ES cell; or

(II) replaced in a first ES cell and a second ES cell, wherein said first ES cell and second ES cell are used respectively to generate a first genetically modified rodent and second genetically modified rodent.

31. The method of claim 30(A)(II) or claim 30(B)(II), wherein the method further comprises breeding the first genetically modified rodent and the second genetically modified rodent to produce the genetically modified rodent of any one of claims 1-27.

32. The method of claim 30(a)(2), wherein:
a humanized MHC I molecule comprises $\alpha 1$, $\alpha 2$, and $\alpha 3$ domains of an HLA class I molecule and at least transmembrane and cytoplasmic domains of an endogenous rodent MHC I molecule, and/or
a humanized MHC II molecule comprises $\alpha 1$ and $\alpha 2$ domains of an HLA class II α molecule, $\beta 1$ and $\beta 2$ domains of an HLA class II β molecule and at least transmembrane and cytoplasmic domains of endogenous rodent MHC II α and MHC II β molecules.
33. The method of claim 30(a)(2), comprising targeting nucleotide sequences encoding an endogenous MHC molecule extracellular domain, endogenous $\beta 2$ microglobulin, and endogenous Ig variable regions and replacing them with an HLA extracellular domain, human $\beta 2$ microglobulin, and human Ig variable regions, respectively.
34. The method of any one of claims 28-33, further comprising administering to the genetically modified rodent the antigenic pMHC.
35. The method of claim 34, wherein the antigenic pMHC is linked to a helper T cell epitope.
36. The method of claim 35, wherein the helper T cell epitope is PADRE.
37. The method of claim 36, wherein PADRE comprises the amino acid sequence set forth in SEQ ID NO: 28.
38. A method of generating a human or humanized antibody that specifically binds an antigenic pMHC, or a nucleic acid sequence encoding the same, comprising maintaining the genetically modified rodent according to any one of claims 1-27 or made according to the method of any one of claims 28-37 in conditions sufficient for the genetically modified rodent to mount an immune response to the antigenic pMHC, wherein the antigenic pMHC comprises a peptide that (i) is heterologous to the genetically modified rodent and (ii) is presented in the context of an HLA molecule or at least the human peptide-binding groove thereof.

39. The method of claim 38, comprising, as a first step, immunizing the genetically modified rodent with the antigenic pMHC.
40. The method of claim 38 or claim 39, wherein the method further comprises boosting the immune response of the immunized genetically modified rodent.
41. The method of claim 39 or claim 40, wherein immunizing and/or boosting comprises administering to the genetically modified rodent the antigenic pMHC linked to a helper T cell epitope.
42. The method of claim 41, wherein the helper T cell epitope comprises PADRE comprising the amino acid sequence set forth as SEQ ID NO:28.
43. A method of obtaining a nucleic acid encoding a human V_H and/or a human V_L , comprising:
isolating from a genetically modified rodent according to any one of claims 22-27 or made according to the method of any one of claims 34-37, a nucleic acid comprising a rearranged human V_H and/or rearranged human V_L nucleotide sequence that encodes the human V_H and/or the human V_L expressed by a lymphocyte of the genetically modified rodent, or a hybridoma produced from the lymphocyte,
wherein the human V_H and/or the human V_L expressed by the lymphocyte, or the hybridoma produced therefrom, associates with its cognate variable domain to form an antigen-binding domain that binds to the antigenic pMHC.
44. The method of claim 43, wherein the obtained rearranged human V_H and/or rearranged human V_L nucleotide sequence comprises at least one somatic hypermutation.
45. A nucleic acid comprising the rearranged human V_H and/or rearranged human V_L nucleotide sequence produced by the method of claim 43 or claim 44.

46. The nucleic acid of claim 45, wherein the nucleic acid further comprises a human Ig constant region gene sequence operably linked to the rearranged human V_H and/or rearranged human V_L nucleotide sequence.
47. The nucleic acid of claim 46, wherein the human Ig heavy chain constant region gene sequence comprises a modification that increases an affinity of a C_H2-C_H3 region of an IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from 5.5 to 6.0, wherein the modification is a mutation in the IgG heavy chain constant region amino acid sequence selected from the group consisting of M428L, N434S, V259I, V308F, N434A, M252Y, S254T, T256E, T250Q, H433K, N434Y, and a combination thereof.
48. A host cell comprising the nucleic acid of any one of claims 45-47.
49. A method of obtaining a cell that expresses a human V_H and/or a human V_L, comprising:
isolating a lymphocyte from a non-human animal according to any one of claims 22-27 or made according to the method of any one of claims 34-37,
wherein the lymphocyte expresses a human V_H and/or a human V_L that forms an antigen-binding domain that binds to the antigenic pMHC.
50. The method of claim 49, further comprising producing a hybridoma from the isolated lymphocyte.
51. An isolated genetically modified rodent cell, comprising:
(a) a nucleotide sequence encoding a human or humanized MHC molecule that comprises at least a human peptide-binding groove of a human leukocyte antigen (HLA) molecule or at least the human peptide-binding groove thereof, and
(b) one or more immunoglobulin (Ig) loci selected from the group consisting of:
(i) an unrearranged or rearranged human or humanized Ig heavy chain locus;
(ii) an unrearranged or rearranged human or humanized Ig light chain locus;
and/or

(iii) an unrearranged or rearranged human or humanized Ig heavy chain locus and an unrearranged or rearranged human or humanized Ig light chain locus, wherein a genetically modified rodent comprising the isolated genetically modified rodent cell is capable of providing human or humanized antibodies comprising a human or humanized antigen-binding domain that binds an antigenic pMHC that comprises:

- (I) a peptide that is heterologous to the genetically modified rodent complexed with
- (II) the HLA molecule or at least the human peptide-binding groove thereof.

52. The isolated genetically modified rodent cell of claim 51, wherein the human or humanized antigen-binding domain comprises human or humanized variable domains.

53. The isolated genetically modified rodent cell of claim 52, wherein at least one of the unrearranged or rearranged human or humanized Ig heavy chain locus and/or the unrearranged or rearranged human or humanized Ig light chain locus is unrearranged.

54. The isolated genetically modified rodent cell of any one of claims 51-53, wherein the isolated cell is obtained according to the method of claim 49 or claim 50.

55. The isolated genetically modified rodent cell of any one of claims 51-54, wherein the isolated genetically modified rodent cell is a somatic cell.

56. The isolated genetically modified rodent cell of claim 55, wherein the somatic cell is a B cell.

57. The isolated genetically modified rodent cell of any one of claims 51-53, wherein the isolated genetically modified rodent cell is a germ cell or an ES cell.

58. An *in vitro* method of making a human V_H and/or a human V_L comprising: expressing, in a cell, a nucleic acid comprising a rearranged human V_H and/or a rearranged human V_L nucleotide sequence that encodes the human V_H and/or the human V_L

expressed by a lymphocyte of a genetically modified rodent according to any one of claims 22-27, or a hybridoma produced from the lymphocyte,

wherein the human V_H and/or the human V_L expressed by the lymphocyte, or the hybridoma produced therefrom, associates with its cognate variable domain to form an antigen-binding domain that binds the antigenic pMHC.

59. The method of claim 58, wherein the nucleic acid further comprises a human Ig constant region gene sequence operably linked to the rearranged human V_H and/or the rearranged human V_L nucleotide sequence.

60. The method of claim 59, wherein the human Ig constant region gene sequence is an Ig heavy chain constant region gene sequence and comprises a modification that increases an affinity of a C_{H2} - C_{H3} region of an IgG heavy chain constant region amino acid sequence to neonatal Fc receptor (FcRn) at a pH ranging from 5.5 to 6.0, wherein the modification is a mutation in the IgG heavy chain constant region amino acid sequence selected from the group consisting of M428L, N434S, V259I, V308F, N434A, M252Y, S254T, T256E, T250Q, H433K, N434Y, and a combination thereof.

61. A human Ig V_H made according to the method of any one of claims 58-60.