CLAIMS

What is claimed is:

1. A genetically modified 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)

(i) an unrearranged human or humanized immunoglobulin heavy chain locus, and/or

(ii) an unrearranged human or humanized immunoglobulin 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:

(i) a human or humanized heavy chain variable domain, expressed from a rearranged immunoglobulin heavy chain variable region locus formed from said unrearranged human or humanized immunoglobulin heavy chain locus; and/or

(ii) a human or humanized light chain variable domain, expressed from a rearranged immunoglobulin light chain variable region locus formed from said unrearranged human or humanized immunoglobulin light chain locus,

wherein the genetically modified rodent is tolerized to:

the human or humanized MHC molecule or at least the human peptide-binding groove thereof, such that it generates a specific B-cell response that generates a human or humanized antibody that:

(A) specifically binds an antigenic peptide-MHC (pMHC) complex 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) does not bind an empty HLA molecule from which the human peptide-binding groove is derived,

when immunized with said antigenic pMHC complex,

wherein the genetically modified rodent further comprises:

(c) said antigenic pMHC complex that comprises:

(i) said peptide that is heterologous to the genetically modified rodent complexed with

(ii) said HLA molecule or at least the human peptide-binding groove thereof, and

(d) said human or humanized antibody that specifically binds said antigenic pMHC complex and does not bind the empty HLA molecule, and

wherein the genetically modified rodent is a rat or a mouse.

2. The genetically modified rodent of claim 1, 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, and/or

wherein the genetically modified rodent comprises:

(a) at an endogenous heavy chain locus:

(i) an unrearranged human or humanized immunoglobulin heavy chain variable region in operable linkage to an endogenous heavy chain constant region;

(ii) a restricted unrearranged human or humanized heavy chain variable region in operable linkage to an endogenous heavy chain constant region;

(iii) a common heavy chain encoding sequence;

(iv) a histidine modified unrearranged human or humanized heavy chain variable region in operable linkage to an endogenous heavy chain constant region;

(v) a heavy chain only immunoglobulin encoding sequence; or

(vi) an unrearranged human or humanized hybrid heavy chain sequence encoding a hybrid immunoglobulin chain;

and/or

(b) at an endogenous light chain locus:

(i) an unrearranged human or humanized immunoglobulin light chain variable region in operable linkage to an endogenous light chain constant region;

(ii) a common light chain encoding sequence;

(iii) a restricted unrearranged human or humanized light chain variable region in operable linkage to an endogenous light chain constant region;

(iv) a histidine modified unrearranged human or humanized light chain variable region in operable linkage to an endogenous light chain constant region; or

(v) a histidine modified rearranged human or humanized light chain variable region in operable linkage to an endogenous light chain constant region.

3. The genetically modified rodent of claim 1, wherein the genetically modified rodent further comprises a functional ADAM6 gene.

4. The genetically modified rodent of claim 3, wherein the functional ADAM6 gene is an endogenous ADAM6 gene.

5. The genetically modified rodent of claim 1, wherein the genetically modified rodent further expresses an exogenous terminal deoxynucleotidyl transferase (TdT) gene.

6. The genetically modified rodent of claim 1, wherein the human or humanized MHC molecule is a human or humanized MHC class I molecule.

7. The genetically modified rodent of claim 6, 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.

8. The genetically modified rodent of claim 6 or claim 7, further comprising in its genome a nucleotide sequence encoding a human or humanized β2 microglobulin,

wherein the genetically modified rodent expresses the human or humanized β2 microglobulin such that the genetically modified rodent is tolerized to the human or humanized β2 microglobulin by itself or in association with the human or humanized MHC class I molecule.

9. The genetically modified rodent of claim 8, wherein the nucleotide sequence encoding the human or humanized β2 microglobulin is at an endogenous β2 microglobulin locus.

10. The genetically modified rodent of claim 1, wherein the human or humanized MHC molecule is a human or humanized MHC class II molecule.

11. The genetically modified rodent of claim 10, wherein the human peptide-binding groove is derived from an α chain and/or 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.

12. The genetically modified rodent of claim 1, 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.

13. The genetically modified rodent of claim 12, wherein the nucleotide sequence encoding a fully human MHC molecule does not disrupt an endogenous rodent MHC locus, and/or wherein the nucleotide sequence encoding a fully human MHC molecule is placed into an endogenous ROSA26 locus.

14. The genetically modified rodent of claim 1, 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 the HLA molecule operably linked to transmembrane and cytoplasmic domains of an endogenous MHC molecule.

15. The genetically modified rodent of claim 14, 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 α1, α2, and α3 domains 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;

(ii) a chimeric human/rodent MHC class I molecule comprising α1, α2, and α3 domains 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,

wherein the endogenous rodent MHC class I molecule comprises an endogenous murine H-2K polypeptide, an endogenous murine H-2D polypeptide, or an endogenous murine H-2L polypeptide;

(iii) a chimeric human/rodent MHC class II molecule comprising an α1 domain and an α2 domain of an HLA class II α polypeptide operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II α molecule, and/or a β1 domain and a β2 domain of an HLA class II β polypeptide operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II β molecule; and/or

(iv) a chimeric human/rodent MHC class II molecule comprising an α1 domain and an α2 domain of an HLA class II α polypeptide operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II α molecule, and/or a β1 domain and a β2 domain of an HLA class II β polypeptide operably linked to the transmembrane and cytoplasmic domains of an endogenous rodent MHC class II β molecule,

wherein the endogenous rodent MHC class II α molecule comprises an endogenous murine H-2A α polypeptide or an endogenous murine H-2E α polypeptide, and/or

wherein the endogenous rodent MHC class II β molecule comprises an endogenous murine H-2A β polypeptide or an endogenous murine H-2E β polypeptide.

16. The genetically modified rodent of claim 1, 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.

17. The genetically modified rodent of claim 1, wherein the genetically modified rodent is a mouse.

18. A method of making the genetically modified rodent of claim 1 comprising:

(1) modifying the genome of a rodent to comprise:

(a) a nucleotide sequence encoding a human or humanized MHC molecule that comprises at least a human peptide-binding groove of an HLA molecule or at least the human peptide-binding groove thereof, and

(b) an unrearranged human or humanized immunoglobulin heavy chain locus and/or an unrearranged human or humanized immunoglobulin light chain locus, and

(2) administering to the rodent an antigenic pMHC complex that comprises a peptide heterologous to the rodent associated with said HLA molecule or at least the human peptide-binding groove thereof,

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 specific B-cell response that generates a human or humanized antibody that (A) specifically binds an antigenic pMHC complex 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) does not bind an empty HLA molecule from which the human peptide-binding groove is derived, when immunized with said antigenic pMHC complex, and

B. capable of expressing from a B-cell said human or humanized antibody which comprises:

(i) a human or humanized heavy chain variable domain, expressed from a rearranged immunoglobulin heavy chain variable region locus formed from said unrearranged human or humanized immunoglobulin heavy chain locus; and/or

(ii) a human or humanized light chain variable domain, expressed from a rearranged immunoglobulin light chain variable region locus formed from said unrearranged human or humanized immunoglobulin light chain locus, and

wherein the rodent is a rat or a mouse.

19. The method of claim 18, 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 polypeptide with a nucleotide sequence encoding a human or humanized MHC I polypeptide to form a nucleotide sequence encoding the human or humanized MHC molecule, and/or

at an endogenous rodent MHC II locus, a nucleotide sequence encoding a rodent MHC II polypeptide -with a nucleotide sequence encoding a human or humanized MHC II polypeptide to form a nucleotide sequence encoding the human or humanized MHC molecule, and

(b)

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

(ii) replacing:

(A) at an endogenous rodent heavy chain locus, an endogenous rodent immunoglobulin heavy chain variable ($V_H$) gene segment with an unrearranged human immunoglobulin heavy chain variable ($V_H$) gene segment, wherein the

unrearranged human $V_H$ gene segment is operably linked to an endogenous heavy chain constant region gene sequence, and/or

(B) at an endogenous rodent light chain locus, an endogenous rodent immunoglobulin light chain variable ($V_L$) gene segment and an endogenous rodent immunoglobulin light chain joining ($J_L$) gene segment with a human immunoglobulin light chain variable ($V_L$) gene segment and a human immunoglobulin light chain joining ($J_L$) gene segment, wherein the human $V_L$ and human $J_L$ gene segments are operably linked to an endogenous 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 human or humanized immunoglobulin heavy chain locus and/or unrearranged human or humanized immunoglobulin light chain locus are:

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

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

(B) the (a)(2) nucleotide sequence encoding the rodent MHC I polypeptide and/or nucleotide sequence encoding the rodent MHC II polypeptide, and the (b)(ii) endogenous rodent $V_H$ gene segment and/or endogenous rodent $V_L$ and endogenous rodent $J_L$ gene segments are:

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

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

20. The method of claim 19, wherein:

(a) item 19(b)(ii)(A) further comprises replacing, at an endogenous rodent heavy chain locus, an endogenous rodent immunoglobulin heavy chain diversity ($D_H$) gene segment and/or an endogenous rodent immunoglobulin heavy chain joining ($J_H$) gene segment with an unrearranged human immunoglobulin heavy chain diversity ($D_H$) gene segment and/or an unrearranged human immunoglobulin heavy chain joining ($J_H$) gene segment, respectively, wherein the unrearranged human $V_H$ gene segment, unrearranged human $D_H$ gene segment and unrearranged human $J_H$ gene segment are operably linked to an endogenous heavy chain constant region gene sequence; and/or

(b) the human $V_L$ gene segment and the human $J_L$ gene segment of item 19(b)(ii)(B) are rearranged to form a $V_L/J_L$ gene sequence.

21. The method of any one of claims 18-20, wherein the antigenic pMHC complex is linked to a helper T cell epitope.

22. The method of claim 21, wherein the helper T cell epitope is PADRE.

23. The method of claim 19(a)(2), wherein:

(a) a humanized MHC I molecule comprises α1, α2, and α3 domains of an HLA class I molecule and at least transmembrane and cytoplasmic domains of an endogenous rodent MHC I polypeptide, and/or wherein a humanized MHC II molecule comprises α1, α2, β1, and β2 domains of an HLA class II molecule and at least transmembrane and cytoplasmic domains of an endogenous rodent MHC II polypeptide; and/or

(b) replacing comprises targeting sequences encoding an endogenous MHC polypeptide extracellular domain, endogenous β2 microglobulin, and endogenous immunoglobulin variable regions and replacing them with sequences encoding a human MHC extracellular domain, human β2 microglobulin, and human immunoglobulin variable regions, respectively.

24. A method of generating a human or humanized antibody that specifically binds an antigenic pMHC complex of interest or a nucleic acid sequence encoding the same comprising maintaining the genetically modified rodent according to claim 1 in conditions sufficient for the genetically modified rodent to mount an immune response to the antigenic pMHC complex of interest, wherein the antigenic pMHC complex of interest comprises a peptide that is

heterologous to the genetically modified rodent and is presented in the context of the HLA molecule or at least the human peptide-binding groove thereof.

25. The method of claim 24, comprising as a first step immunizing the genetically modified rodent with the antigenic pMHC complex of interest.

26. The method of claim 25, wherein the method comprises further boosting the immune response of the immunized genetically modified rodent,

wherein the method comprises further boosting the immune response of the immunized genetically modified rodent and wherein immunizing and/or boosting comprises administering to the genetically modified rodent the antigenic pMHC complex of interest linked to a helper T cell epitope, and/or

wherein the method comprises further boosting the immune response of the immunized genetically modified rodent and wherein immunizing and/or boosting comprises administering to the genetically modified rodent the antigenic pMHC complex of interest linked to a helper T cell epitope comprising PADRE set forth as SEQ ID NO:28.

27. A method of obtaining a nucleic acid encoding a human immunoglobulin heavy chain variable domain and/or a human immunoglobulin light chain variable domain, comprising:

isolating from a genetically modified rodent according to claim 1, a nucleic acid comprising a rearranged human immunoglobulin variable region gene sequence that encodes a human immunoglobulin variable domain expressed by a lymphocyte of the genetically modified rodent, or a hybridoma produced from the lymphocyte,

wherein the human immunoglobulin variable domain expressed by the lymphocyte, or hybridoma produced therefrom, associates with its cognate variable domain to form an antigen-binding domain specific for the antigenic pMHC complex.

28. The method of claim 27, wherein the obtained rearranged human immunoglobulin variable region gene sequence comprises at least one somatic hypermutation.

29. A method of obtaining a cell that expresses a human immunoglobulin heavy chain variable domain and/or a human immunoglobulin light chain variable domain comprising:

isolating a lymphocyte from a genetically modified rodent according to claim 1,

wherein the lymphocyte expresses a human immunoglobulin variable domain that forms an antigen-binding domain specific for the antigenic pMHC complex.

30. The method of claim 29, further comprising producing a hybridoma from the isolated lymphocyte.

31. An *in vitro* method of making a human immunoglobulin variable domain comprising:

expressing, in a cell, a first nucleic acid comprising a rearranged human immunoglobulin variable region gene sequence that encodes a human immunoglobulin variable domain expressed by a lymphocyte of a genetically modified rodent according to claim 1, or a hybridoma produced from the lymphocyte,

wherein the human immunoglobulin variable domain expressed by the lymphocyte, or the hybridoma produced therefrom, associates with its cognate variable domain to form an antigen-binding domain specific for the antigenic pMHC complex.

32. The method of claim 31, wherein the first nucleic acid further comprises a human immunoglobulin constant region gene sequence operably linked to the rearranged human immunoglobulin variable region gene sequence.

33. The method of claim 32, wherein the human immunoglobulin constant region gene sequence is a 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.