COMMON LIGHT CHAIN MOUSE

FIELD OF INVENTION

[0001] A genetically modified mouse is provided that expresses antibodies having a common human variable/mouse constant light chain associated with diverse human variable/mouse constant heavy chains. A method for making a human bispecific antibody from human variable region gene sequences of B cells of the mouse is provided.

BACKGROUND

[0002] Antibodies typically comprise a homodimeric heavy chain component, wherein each heavy chain monomer is associated with an identical light chain. Antibodies having a heterodimeric heavy chain component (*e.g.*, bispecific antibodies) are desirable as therapeutic antibodies. But making bispecific antibodies having a suitable light chain component that can satisfactorily associate with each of the heavy chains of a bispecific antibody has proved problematic.

[0003] In one approach, a light chain might be selected by surveying usage statistics for all light chain variable domains, identifying the most frequently employed light chain in human antibodies, and pairing that light chain in vitro with the two heavy chains of differing specificity.

[0004] In another approach, a light chain might be selected by observing light chain sequences in a phage display library (*e.g.,* a phage display library comprising human light chain variable region sequences, *e.g.,* a human ScFv library) and selecting the most commonly used light chain variable region from the library. The light chain can then be tested on the two different heavy chains of interest.

[0005] In another approach, a light chain might be selected by assaying a phage display library of light chain variable sequences using the heavy chain variable sequences of both heavy chains of interest as probes. A light chain that associates with both heavy chain variable sequences might be selected as a light chain for the heavy chains.

[0006] In another approach, a candidate light chain might be aligned with the heavy chains' cognate light chains, and modifications are made in the light chain to more closely match sequence characteristics common to the cognate light chains of both heavy chains. If the chances of immunogenicity need to be minimized, the modifications preferably result in sequences that are present in known human light chain sequences, such that proteolytic processing is unlikely to generate a T cell epitope based on parameters and methods known in the art for assessing the likelihood of immunogenicity (*i.e., in silico* as well as wet assays).

[0007] All of the above approaches rely on in vitro methods that subsume a number of a priori restraints, *e.g.,* sequence identity, ability to associate with specific pre-selected heavy chains, *etc.* There is a need in the art for compositions and methods that do not rely on manipulating in vitro conditions, but that instead employ more biologically sensible approaches to making human epitope-binding proteins that include a common light chain.

BRIEF DESCRIPTION OF THE FIGURES

[0008] FIG. 1 illustrates a targeting strategy for replacing endogenous mouse immunoglobulin light chain variable region gene segments with a human Vκ1-39Jκ5 gene region.

[0009] FIG. 2 illustrates a targeting strategy for replacing endogenous mouse immunoglobulin light chain variable region gene segments with a human Vκ3-20Jκ1 gene region.

[0010] FIG. 3 illustrates a targeting strategy for replacing endogenous mouse immunoglobulin light chain variable region gene segments with a human VpreB/Jλ5 gene region.

SUMMARY

[0011] Genetically modified mice that express human immunoglobulin heavy and light chain variable domains, wherein the mice have a limited light chain variable repertoire, are provided. A biological system for generating a human light chain variable domain that associates and expresses with a diverse repertoire of affinity-matured human heavy chain variable domains is provided. Methods for making binding proteins comprising immunoglobulin variable domains are provided, comprising immunizing mice that have a limited immunoglobulin light chain repertoire with an antigen of interest, and employing an immunoglobulin variable region gene sequence of the mouse in a binding protein that specifically binds the antigen of interest. Methods include methods for making human immunoglobulin heavy chain variable domains suitable for use in making multi-specific antigen-binding proteins.

[0012] Genetically engineered mice are provided that select suitable affinity-matured human immunoglobulin heavy chain variable domains derived from a repertoire of unrearranged human heavy chain variable region gene segments, wherein the affinity-matured human heavy chain variable domains associate and express with a single human light chain variable domain derived from one human light chain variable region gene segment. Genetically engineered mice that present a choice of two human light chain variable region gene segments are also provided.

[0013] Genetically engineered mice are provided that express a limited repertoire of human light chain variable domains, or a single human light chain variable domain, from a limited repertoire of human light chain variable region gene segments. The mice are genetically engineered to include a single unrearranged human light chain variable region gene segment (or two human light chain variable region gene segments) that rearranges to form a rearranged human light chain variable region gene (or two rearranged light chain variable region genes) that express a single light chain (or that express either or both of two light chains). The rearranged human light chain variable domains are capable of pairing with a plurality of affinity-matured human heavy chains selected by the mice, wherein the heavy chain variable regions specifically bind different epitopes.

[0014] In one aspect, a genetically modified mouse is provided that comprises a single human immunoglobulin light chain variable (VL) region gene segment that is capable of rearranging and encoding a human VL domain of an immunoglobulin light chain. In another aspect, the mouse comprises no more than two human VL gene segments that are capable of rearranging and encoding a human VL domain of an immunoglobulin light chain.

[0015] In one aspect, a genetically modified mouse is provided that comprises a single rearranged (V/J) human immunoglobulin light chain variable (VL) region segment (*i.e.,* a V/J segment) that encodes a human VL domain of an immunoglobulin light chain. In another aspect, the mouse comprises no more than two rearranged human VL gene segments that are capable of encoding a human VL domain of an immunoglobulin light chain.

[0016] In one embodiment, the VL gene segment is a human Vκ1-39Jκ5 gene segment or a human Vκ3-20Jκ1 gene segment. In one embodiment, the mouse has both a human Vκ1-39Jκ5 gene segment and a human Vκ3-20Jκ1 gene segment.

[0017] In one embodiment, the human VL gene segment is operably linked to a human or mouse leader sequence. In one embodiment, the leader sequence is a mouse leader sequence. In a specific embodiment, the mouse leader sequence is a mouse Vκ3-7 leader sequence.

[0018] In one embodiment, the VL gene segment is operably linked to an immunoglobulin promoter sequence. In one embodiment, the promoter sequence is a human promoter sequence. In a specific embodiment, the human immunoglobulin promoter is a Vκ3-15 promoter.

[0019] In one embodiment, the genetically modified mouse comprises a VL locus that does not comprise an endogenous mouse VL gene segment that is capable of rearranging to form an immunoglobulin light chain gene, wherein the VL locus comprises a single human VL gene segment that is capable of rearranging to encode a VL region of a light chain gene. In

a specific embodiment, the human VL gene segment is a human Vκ1-39Jκ5 gene segment or a human Vκ3-20Jκ1 gene segment.

[0020] In one embodiment, the VL locus comprises a leader sequence flanked 5' (with respect to transcriptional direction of the VL gene segment) with a human immunoglobulin promoter and flanked 3' with a human VL gene segment that rearranges and encodes VL domain of a reverse chimeric light chain comprising an endogenous mouse light chain constant region (CL). In a specific embodiment, the VL gene segment is at the mouse kappa (κ) VL locus, and the mouse CL is a mouse κ CL.

[0021] In one embodiment, the mouse comprises a nonfunctional lambda (λ) immunoglobulin light chain locus. In a specific embodiment, the λ locus comprises a deletion of one or more sequences of the locus, wherein the one or more deletions renders the λ locus incapable of rearranging to form a light chain gene. In another embodiment all or substantially all of the VL gene segments of the λ locus are deleted.

[0022] In one embodiment, the VL locus of the modified mouse is a κ locus, and the κ locus comprises a mouse κ intronic enhancer, a mouse κ 3' enhancer, or both an intronic enhancer and a 3' enhancer.

[0023] In one embodiment, mouse makes a light chain that comprises a somatically mutated VL domain derived from a human VL gene segment. In one embodiment, the light chain comprises a somatically mutated VL domain derived from a human VL gene segment, and a mouse κ CL region. In one embodiment, the mouse does not express a λ light chain.

[0024] In one embodiment, the genetically modified mouse is capable of somatically hypermutating the human VL region sequence. In a specific embodiment, the mouse comprises a cell that comprises a rearranged immunoglobulin light chain gene derived from the human VL gene segment that is capable of rearranging and encoding a VL domain, and the rearranged immunoglobulin light chain gene comprises a somatically mutated VL domain.

[0025] In one embodiment, the mouse comprises a cell that expresses a light chain comprising a somatically mutated human VL domain linked to a mouse κ CL, wherein the light chain associates with a heavy chain comprising a somatically mutated VH domain derived from a human VH gene segment and wherein the heavy chain comprises a mouse heavy chain constant region (CH).

[0026] In one embodiment, the mouse comprises a replacement of endogenous mouse VH gene segments with one or more human VH gene segments, wherein the human VH gene segments are operably linked to a mouse CH region gene, such that the mouse rearranges the human VH gene segments and expresses a reverse chimeric immunoglobulin heavy chain that comprises a human VH domain and a mouse CH. In one embodiment, 90-100%

of unrearranged mouse VH gene segments are replaced with at least one unrearranged human VH gene segment. In a specific embodiment, all or substantially all of the endogenous mouse VH gene segments are replaced with at least one unrearranged human VH gene segment. In one embodiment, the replacement is with at least 19, at least 39, or at least 80 or 81 unrearranged human VH gene segments. In one embodiment, the replacement is with at least 12 functional unrearranged human VH gene segments, at least 25 functional unrearranged human VH gene segments, or at least 43 functional unrearranged human VH gene segments. In one embodiment, the mouse comprises a replacement of all mouse D and J segments with at least one unrearranged human D segment and at least one unrearranged human J segment. In one embodiment, the at least one unrearranged human D segment is selected from D1-7, D1-26, D3-3, D3-10, D3-16, D3-22, D5-5, D5-12, D6-6, D6-13, D7-27, and a combination thereof. In one embodiment, the at least one unrearranged human J segment is selected from J1, J3, J4, J5, J6, and a combination thereof. In a specific embodiment, the one or more human VH gene segment is selected from a 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, a 6-1 human VH gene segment, and a combination thereof.

[0027] In one embodiment, the mouse comprises a B cell that expresses a binding protein that specifically binds an antigen of interest, wherein the binding protein comprises a light chain derived from a human Vκ1-39/Jκ5 rearrangement or a human Vκ3-20/Jκ1 rearrangement, and wherein the cell comprises a rearranged immunoglobulin heavy chain gene derived from a rearrangement of human gene segments selected from a VH2-5, VH3-23, VH3-30, VH 4-39, VH4-59, and VH5-51 gene segment. In one embodiment, the one or more human VH gene segments are rearranged with a human heavy chain J gene segment selected from J1, J3, J4, J5, and J6. In one embodiment, the one or more human VH and J gene segments are rearranged with a human D gene segment selected from D1-7, D1-26, D3-3, D3-10, D3-16, D3-22, D5-5, D5-12, D6-6, D6-13, and D7-27. In a specific embodiment, the light chain gene has 1, 2, 3, 4, or 5 or more somatic hypermutations.

[0028] In one embodiment, the mouse comprises a B cell that comprises a rearranged immunoglobulin heavy chain variable region gene sequence comprising a VH, JH, and DH gene segment selected from VH 2-5 + JH1 + D6-6, VH3-23 + JH4 + D3, VH3-23 + JH4 + D3-10, VH3-30 + JH1 + D6-6, VH3-30 +JH3 + D6-6, VH3-30 + JH4 + D1-7, VH3-30 + JH4 + D5-12, VH3-30 + JH4 + D6-13, VH3-30 + JH4 + D6-6, VH3-30 + JH4 + D7-27, VH3-30 + JH5 + D3-22, VH3-30 + JH5 + D6-6, VH3-30 + JH5 + D7-27, VH4-39 + JH3 + D1-26, VH4-59 + JH3 + D3-16, VH4-59 + JH3 + D3-22, VH4-59 + JH4 + D3-16, VH5-51 + JH3 + D5-5, VH5-51 + JH5 + D6-13, and VH5-51 + JH6 + D3-16. In a specific embodiment, the B cell expresses a binding protein comprising a human immunoglobulin heavy chain variable

region fused with a mouse heavy chain constant region, and a human immunoglobulin light chain variable region fused with a mouse light chain constant region.

[0029] In one embodiment, the human VL gene segment is a human Vκ1-39Jκ5 gene segment, and the mouse expresses a reverse chimeric light chain comprising (i) a VL domain derived from the human VL gene segment and (ii) a mouse CL; wherein the light chain is associated with a reverse chimeric heavy chain comprising (i) a mouse CH and (ii) a somatically mutated human VH domain derived from a human VH gene segment selected from a 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, and 6-1 human VH gene segment, and a combination thereof. In one embodiment, the mouse expresses a light chain that is somatically mutated. In one embodiment the CL is a mouse κ CL.

[0030] In one embodiment, the human VL gene segment is a human Vκ3-20Jκ1 gene segment, and the mouse expresses a reverse chimeric light chain comprising (i) a VL domain derived from the human VL gene segment, and (ii) a mouse CL; wherein the light chain is associated with a reverse chimeric heavy chain comprising (i) a mouse CH, and (ii) a somatically mutated human VH derived from a human VH gene segment selected from a 1-2, 2-5, 3-7, 3-9, 3-11, 3-20, 3-23, 3-30, 3-33, 4-59, and 5-51 human VH gene segment, and a combination thereof. In one embodiment, the mouse expresses a light chain that is somatically mutated. In one embodiment the CL is a mouse κ CL.

[0031] In one embodiment, the mouse comprises both a human Vκ1-39Jκ5 gene segment and a human Vκ3-20Jκ1 gene segment, and the mouse expresses a reverse chimeric light chain comprising (i) a VL domain derived from a human Vκ1-39Jκ5 gene segment or a human Vκ3-20Jκ1 gene segment, and (ii) a mouse CL; wherein the light chain is associated with a reverse chimeric heavy chain comprising (i) a mouse CH, and (ii) a somatically mutated human VH derived from a human VH gene segment selected from a 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, a 6-1 human VH gene segment, and a combination thereof. In one embodiment, the mouse expresses a light chain that is somatically mutated. In one embodiment the CL is a mouse κ CL.

[0032] In one embodiment, 90-100% of the endogenous unrearranged mouse VH gene segments are replaced with at least one unrearranged human VH gene segment. In a specific embodiment, all or substantially all of the endogenous unrearranged mouse VH gene segments are replaced with at least one unrearranged human VH gene segment. In one embodiment, the replacement is with at least 18, at least 39, at least 80, or 81 unrearranged human VH gene segments. In one embodiment, the replacement is with at least 12 functional unrearranged human VH gene segments, at least 25 functional

unrearranged human VH gene segments, or at least 43 unrearranged human VH gene segments.

[0033] In one embodiment, the genetically modified mouse is a C57BL strain, in a specific embodiment selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, C57BL/Ola. In a specific embodiment, the genetically modified mouse is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In another specific embodiment, the mouse is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In a specific embodiment, the 129 strain of the mix is a 129S6 (129/SvEvTac) strain.

[0034] In one embodiment, the mouse expresses a reverse chimeric antibody comprising a light chain that comprises a mouse κ CL and a somatically mutated human VL domain derived from a human Vκ1-39Jκ5 gene segment or a human Vκ3-20Jκ1 gene segment, and a heavy chain that comprises a mouse CH and a somatically mutated human VH domain derived from a human VH gene segment selected from a 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, and a 6-1 human VH gene segment, wherein the mouse does not express a fully mouse antibody and does not express a fully human antibody. In one embodiment the mouse comprises a κ light chain locus that comprises a replacement of endogenous mouse κ VL gene segments with the human Vκ1-39Jκ5 gene segment or the human Vκ3-20Jκ1 gene segment, and comprises a replacement of all or substantially all endogenous mouse VH gene segments with a complete or substantially complete repertoire of human VH gene segments.

[0035] In one aspect, a mouse cell is provided that is isolated from a mouse as described herein. In one embodiment, the cell is an ES cell. In one embodiment, the cell is a lymphocyte. In one embodiment, the lymphocyte is a B cell. In one embodiment, the B cell expresses a chimeric heavy chain comprising a variable domain derived from a human gene segment; and a light chain derived from a rearranged human Vκ1-39/J segment, rearranged human Vκ3-20/J segment, or a combination thereof; wherein the heavy chain variable domain is fused to a mouse constant region and the light chain variable domain is fused to a mouse or a human constant region.

[0036] In one aspect, a hybridoma is provided, wherein the hybridoma is made with a B cell of a mouse as described herein. In a specific embodiment, the B cell is from a mouse as described herein that has been immunized with an immunogen comprising an epitope of interest, and the B cell expresses a binding protein that binds the epitope of interest, the binding protein has a somatically mutated human VH domain and a mouse CH, and has a human VL domain derived from a human Vκ1-39Jκ5 or a human Vκ3-20Jκ1 gene segment and a mouse CL.

[0037] In one aspect, a mouse embryo is provided, wherein the embryo comprises a donor ES cell that is derived from a mouse as described herein.

[0038] In one aspect, a targeting vector is provided, comprising, from 5' to 3' in transcriptional direction with reference to the sequences of the 5' and 3' mouse homology arms of the vector, a 5' mouse homology arm, a human or mouse immunoglobulin promoter, a human or mouse leader sequence, and a human LCVR gene segment selected from a human Vκ1-39Jκ5 or a human Vκ3-20Jκ1 gene segment, and a 3' mouse homology arm. In one embodiment, the 5' and 3' homology arms target the vector to a sequence 5' with respect to an enhancer sequence that is present 5' and proximal to the mouse κ constant region gene. In one embodiment, the promoter is a human immunoglobulin variable region gene segment promoter. In a specific embodiment, the promoter is a human Vκ3-15 promoter. In one embodiment, the leader sequence is a mouse leader sequence. In a specific embodiment, the mouse leader sequence is a mouse Vκ3-7 leader sequence.

[0039] In one aspect, a targeting vector is provided as described above, but in place of the 5' mouse homology arm the human or mouse promoter is flanked 5' with a site-specific recombinase recognition site (SRRS), and in place of the 3' mouse homology arm the human LCVR gene segment is flanked 3' with an SRRS.

[0040] In one aspect, a reverse chimeric antibody made by a mouse as described herein, wherein the reverse chimeric antibody comprises a light chain comprising a mouse CL and a human VL, and a heavy chain comprising a human VH and a mouse CH.

[0041] In one aspect, a method for making an antibody is provided, comprising expressing in a single cell (a) a first VH gene sequence of an immunized mouse as described herein fused with a human CH gene sequence; (b) a VL gene sequence of an immunized mouse as described herein fused with a human CL gene sequence; and, (c) maintaining the cell under conditions sufficient to express a fully human antibody, and isolating the antibody. In one embodiment, the cell comprises a second VH gene sequence of a second immunized mouse as described herein fused with a human CH gene sequence, the first VH gene sequence encodes a VH domain that recognizes a first epitope, and the second VH gene sequence encodes a VH domain that recognizes a second epitope, wherein the first epitope and the second epitope are not identical.

[0042] In one aspect, a method for making an epitope-binding protein is provided, comprising exposing a mouse as described herein with an immunogen that comprises an epitope of interest, maintaining the mouse under conditions sufficient for the mouse to generate an immunoglobulin molecule that specifically binds the epitope of interest, and isolating the immunoglobulin molecule that specifically binds the epitope of interest; wherein the epitope-binding protein comprises a heavy chain that comprises a somatically mutated

human VH and a mouse CH, associated with a light chain comprising a mouse CL and a human VL derived from a human Vκ1-39 Jκ5 or a human Vκ3-20 Jκ1 gene segment.

[0043] In one aspect, a cell that expresses an epitope-binding protein is provided, wherein the cell comprises: (a) a human VL nucleotide sequence encoding a human VL domain derived from a human Vκ1-39Jκ5 or a human Vκ3-20Jκ1 gene segment, wherein the human VL nucleotide sequence is fused (directly or through a linker) to a human immunoglobulin light chain constant domain cDNA sequence (*e.g.,* a human κ constant domain DNA sequence); and, (b) a first human VH nucleotide sequence encoding a human VH domain derived from a first human VH nucleotide sequence, wherein the first human VH nucleotide sequence is fused (directly or through a linker) to a human immunoglobulin heavy chain constant domain cDNA sequence; wherein the epitope-binding protein recognizes a first epitope. In one embodiment, the epitope-binding protein binds the first epitope with a dissociation constant of lower than 10^{-6} M, lower than 10^{-8} M, lower than 10^{-9} M, lower than 10^{-10} M, lower than 10^{-11} M, or lower than 10^{-12} M.

[0044] In one embodiment, the cell comprises a second human VH nucleotide sequence encoding a second human VH domain, wherein the second human VH sequence is fused (directly or through a linker) to a human immunoglobulin heavy chain constant domain cDNA sequence, and wherein the second human VH domain does not specifically recognize the first epitope (*e.g.,* displays a dissociation constant of, *e.g.,* 10^{-6} M, 10^{-5} M, 10^{-4} M, or higher), and wherein the epitope-binding protein recognizes the first epitope and the second epitope, and wherein the first and the second immunoglobulin heavy chains each associate with an identical light chain of (a).

[0045] In one embodiment, the second VH domain binds the second epitope with a dissociation constant that is lower than 10^{-6} M, lower than 10^{-7} M, lower than 10^{-8} M, lower than 10^{-9} M, lower than 10^{-10} M, lower than 10^{-11} M, or lower than 10^{-12} M.

[0046] In one embodiment, the epitope-binding protein comprises a first immunoglobulin heavy chain and a second immunoglobulin heavy chain, each associated with an identical light chain derived from a human VL gene segment selected from a human Vκ1-39Jκ5 or a human Vκ3-20Jκ1 gene segment, wherein the first immunoglobulin heavy chain binds a first epitope with a dissociation constant in the nanomolar to picomolar range, the second immunoglobulin heavy chain binds a second epitope with a dissociation constant in the nanomolar to picomolar range, the first epitope and the second epitope are not identical, the first immunoglobulin heavy chain does not bind the second epitope or binds the second epitope with a dissociation constant weaker than the micromolar range (*e.g.,* the millimolar range), the second immunoglobulin heavy chain does not bind the first epitope or binds the first epitope with a dissociation constant weaker than the micromolar range (*e.g.,* the

millimolar range), and one or more of the VL, the VH of the first immunoglobulin heavy chain, and the VH of the second immunoglobulin heavy chain, are somatically mutated.

[0047] In one embodiment, the first immunoglobulin heavy chain comprises a protein A-binding residue, and the second immunoglobulin heavy chain lacks the protein A-binding residue.

[0048] In one embodiment, the cell is selected from CHO, COS, 293, HeLa, and a retinal cell expressing a viral nucleic acid sequence (*e.g.,* a PERC.6™ cell).

[0049] In one aspect, a reverse chimeric antibody is provided, comprising a human VH and a mouse heavy chain constant domain, a human VL and a mouse light chain constant domain, wherein the antibody is made by a process that comprises immunizing a mouse as described herein with an immunogen comprising an epitope, and the antibody specifically binds the epitope of the immunogen with which the mouse was immunized. In one embodiment, the VL domain is somatically mutated. In one embodiment the VH domain is somatically mutated. In one embodiment, both the VL domain and the VH domain are somatically mutated. In one embodiment, the VL is linked to a mouse κ constant domain.

[0050] In one aspect, a mouse is provided, comprising human heavy chain variable gene segments replacing all or substantially all mouse heavy chain variable gene segments at the endogenous mouse locus; no more than one or two human light chain variable gene segments selected from a rearranged Vκ1-39/J and a rearranged Vκ3-20/J segment or a combination thereof, replacing all mouse light chain variable gene segments; wherein the human heavy chain variable gene segments are linked to a mouse constant gene, and the human light chain variable gene segment(s) is linked to a human or mouse constant gene.

[0051] In one aspect, a mouse ES cell comprising a replacement of all or substantially all mouse heavy chain variable gene segments with human heavy chain variable gene segments, and no more than one or two rearranged human light chain V/J segments, wherein the human heavy chain variable gene segments are linked to a mouse immunoglobulin heavy chain constant gene, and the human light chain V/J segments are linked to a mouse or human immunoglobulin light chain constant gene. In a specific embodiment, the light chain constant gene is a mouse constant gene.

[0052] In one aspect, an antigen-binding protein made by a mouse as described herein is provided. In a specific embodiment, the antigen-binding protein comprises a human immunoglobulin heavy chain variable region fused with a mouse constant region, and a human immunoglobulin light chain variable region derived from a Vκ1-39 gene segment or a Vκ3-20 gene segment, wherein the light chain constant region is a mouse constant region.

[0053] In one aspect, a fully human antigen-binding protein made from an immunoglobulin variable region gene sequence from a mouse as described herein is provided, wherein the

antigen-binding protein comprises a fully human heavy chain comprising a human variable region derived from a sequence of a mouse as described herein, and a fully human light chain comprising a Vκ1-39 or a Vκ3-20 variable region. In one embodiment, the light chain variable region comprises one to five somatic mutations. In one embodiment, the light chain variable region is a cognate light chain variable region that is paired in a B cell of the mouse with the heavy chain variable region.

[0054] In one embodiment, the fully human antigen-binding protein comprises a first heavy chain and a second heavy chain, wherein the first heavy chain and the second heavy chain comprise non-identical variable regions independently derived from a mouse as described herein, and wherein each of the first and second heavy chains express from a host cell associated with a human light chain derived from a Vκ1-39 gene segment or a Vκ3-20 gene segment. In one embodiment, the first heavy chain comprises a first heavy chain variable region that specifically binds a first epitope of a first antigen, and the second heavy chain comprises a second heavy chain variable region that specifically binds a second epitope of a second antigen. In a specific embodiment, the first antigen and the second antigen are different. In a specific embodiment, the first antigen and the second antigen are the same, and the first epitope and the second epitope are not identical; in a specific embodiment, binding of the first epitope by a first molecule of the binding protein does not block binding of the second epitope by a second molecule of the binding protein.

[0055] In one aspect, a fully human binding protein derived from a human immunoglobulin sequence of a mouse as described herein comprises a first immunoglobulin heavy chain and a second immunoglobulin heavy chain, wherein the first immunoglobulin heavy chain comprises a first variable region that is not identical to a variable region of the second immunoglobulin heavy chain, and wherein the first immunoglobulin heavy chain comprises a wild-type protein A binding determinant, and the second heavy chain lacks a wild-type protein A binding determinant. In one embodiment, the first immunoglobulin heavy chain binds protein A under isolation conditions, and the second immunoglobulin heavy chain does not bind protein A or binds protein A at least 10-fold, a hundred-fold, or a thousand-fold weaker than the first immunoglobulin heavy chain binds protein A under isolation conditions. In a specific embodiment, the first and the second heavy chains are IgG1 isotypes, wherein the second heavy chain comprises a modification selected from 95R (EU 435R), 96F (EU 436F), and a combination thereof, and wherein the first heavy chain lacks such modification.

[0056] In one aspect, a method for making a bispecific antigen-binding protein is provided, comprising exposing a first mouse as described herein to a first antigen of interest that comprises a first epitope, exposing a second mouse as described herein to a second antigen of interest that comprises a second epitope, allowing the first and the second mouse

to each mount immune responses to the antigens of interest, identifying in the first mouse a first human heavy chain variable region that binds the first epitope of the first antigen of interest, identifying in the second mouse a second human heavy chain variable region that binds the second epitope of the second antigen of interest, making a first fully human heavy chain gene that encodes a first heavy chain that binds the first epitope of the first antigen of interest, making a second fully human heavy chain gene that encodes a second heavy chain that binds the second epitope of the second antigen of interest, expressing the first heavy chain and the second heavy chain in a cell that expresses a single fully human light chain derived from a human Vκ1-39 or a human Vκ3-20 gene segment to form a bispecific antigen-binding protein, and isolating the bispecific antigen-binding protein.

[0057] In one embodiment, the first antigen and the second antigen are not identical.

[0058] In one embodiment, the first antigen and the second antigen are identical, and the first epitope and the second epitope are not identical. In one embodiment, binding of the first heavy chain variable region to the first epitope does not block binding of the second heavy chain variable region to the second epitope.

[0059] In one embodiment, the first antigen is selected from a soluble antigen and a cell surface antigen (*e.g.*, a tumor antigen), and the second antigen comprises a cell surface receptor. In a specific embodiment, the cell surface receptor is an immunoglobulin receptor. In a specific embodiment, the immunoglobulin receptor is an Fc receptor. In one embodiment, the first antigen and the second antigen are the same cell surface receptor, and binding of the first heavy chain to the first epitope does not block binding of the second heavy chain to the second epitope.

[0060] In one embodiment, the light chain variable domain of the light chain comprises 2 to 5 somatic mutations. In one embodiment, the light chain variable domain is a somatically mutated cognate light chain expressed in a B cell of the first or the second immunized mouse with either the first or the second heavy chain variable domain.

[0061] In one embodiment, the first fully human heavy chain bears an amino acid modification that reduces its affinity to protein A, and he second fully human heavy chain does not comprise a modification that reduces its affinity to protein A.

[0062] In one aspect, an antibody or a bispecific antibody comprising a human heavy chain variable domain made in accordance with the invention is provided. In another aspect, use of a mouse as described herein to make a fully human antibody or a fully human bispecific antibody is provided.

[0063] Any of the embodiments and aspects described herein can be used in conjunction with one another, unless otherwise indicated or apparent from the context. Other

embodiments will become apparent to those skilled in the art from a review of the ensuing description.

DETAILED DESCRIPTION

[0064] This invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[0065] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described. All publications mentioned are hereby incorporated by reference.

[0066] The term "antibody", as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain comprises a heavy chain variable (VH) region and a heavy chain constant region (CH). The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable (VL) region and a light chain constant region (CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (heavy chain CDRs may be abbreviated as HCDR1, HCDR2 and HCDR3; light chain CDRs may be abbreviated as LCDR1, LCDR2 and LCDR3. The term "high affinity" antibody refers to an antibody that has a K_D with respect to its target epitope about of 10^{-9} M or lower (*e.g.,* about 1 x 10^{-9} M, 1 x 10^{-10} M, 1 x 10^{-11} M, or about 1 x 10^{-12} M). In one embodiment, K_D is measured by surface plasmon resonance, *e.g.,* BIACORE™; in another embodiment, K_D is measured by ELISA.

[0067] The phrase "bispecific antibody" includes an antibody capable of selectively binding two or more epitopes. Bispecific antibodies generally comprise two nonidentical heavy chains, with each heavy chain specifically binding a different epitope—either on two different molecules (*e.g.,* different epitopes on two different immunogens) or on the same molecule (*e.g.,* different epitopes on the same immunogen). If a bispecific antibody is capable of selectively binding two different epitopes (a first epitope and a second epitope), the affinity of the first heavy chain for the first epitope will generally be at least one to two or three or four

or more orders of magnitude lower than the affinity of the first heavy chain for the second epitope, and vice versa. Epitopes specifically bound by the bispecific antibody can be on the same or a different target (*e.g.,* on the same or a different protein). Bispecific antibodies can be made, for example, by combining heavy chains that recognize different epitopes of the same immunogen. For example, nucleic acid sequences encoding heavy chain variable sequences that recognize different epitopes of the same immunogen can be fused to nucleic acid sequences encoding the same or different heavy chain constant regions, and such sequences can be expressed in a cell that expresses an immunoglobulin light chain. A typical bispecific antibody has two heavy chains each having three heavy chain CDRs, followed by (N-terminal to C-terminal) a CH1 domain, a hinge, a CH2 domain, and a CH3 domain, and an immunoglobulin light chain that either does not confer epitope-binding specificity but that can associate with each heavy chain, or that can associate with each heavy chain and that can bind one or more of the epitopes bound by the heavy chain epitope-binding regions, or that can associate with each heavy chain and enable binding or one or both of the heavy chains to one or both epitopes.

[0068] The term "cell" includes any cell that is suitable for expressing a recombinant nucleic acid sequence. Cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (*e.g.,* strains of *E. coli, Bacillus spp., Streptomyces spp., etc.*), mycobacteria cells, fungal cells, yeast cells (*e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.*), plant cells, insect cells (*e.g.,* SF-9, SF-21, baculovirus-infected insect cells, *Trichoplusia ni, etc.*), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (*e.g.,* CHO K1, DXB-11 CHO, Veggie-CHO), COS (*e.g.,* COS-7), retinal cell, Vero, CV1, kidney (*e.g.,* HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (*e.g.,* BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, *e.g.,* a retinal cell that expresses a viral gene (*e.g.,* a PER.C6™ cell).

[0069] The phrase "complementarity determining region," or the term "CDR," includes an amino acid sequence encoded by a nucleic acid sequence of an organism's immunoglobulin genes that normally (*i.e.,* in a wild-type animal) appears between two framework regions in a variable region of a light or a heavy chain of an immunoglobulin molecule (*e.g.,* an antibody or a T cell receptor). A CDR can be encoded by, for example, a germline sequence or a rearranged or unrearranged sequence, and, for example, by a naive or a mature B cell or a T cell. A CDR can be somatically mutated (*e.g.,* vary from a sequence encoded in an

animal's germline), humanized, and/or modified with amino acid substitutions, additions, or deletions. In some circumstances (*e.g.,* for a CDR3), CDRs can be encoded by two or more sequences (*e.g.,* germline sequences) that are not contiguous (*e.g.,* in an unrearranged nucleic acid sequence) but are contiguous in a B cell nucleic acid sequence, *e.g.,* as the result of splicing or connecting the sequences (*e.g.,* V-D-J recombination to form a heavy chain CDR3).

[0070] The term "conservative," when used to describe a conservative amino acid substitution, includes substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (*e.g.,* charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a variable region to specifically bind a target epitope with a desired affinity. Examples of groups of amino acids that have side chains with similar chemical properties include aliphatic side chains such as glycine, alanine, valine, leucine, and isoleucine; aliphatic-hydroxyl side chains such as serine and threonine; amide-containing side chains such as asparagine and glutamine; aromatic side chains such as phenylalanine, tyrosine, and tryptophan; basic side chains such as lysine, arginine, and histidine; acidic side chains such as aspartic acid and glutamic acid; and, sulfur-containing side chains such as cysteine and methionine. Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine, phenylalanine/tyrosine, lysine/arginine, alanine/valine, glutamate/aspartate, and asparagine/glutamine. In some embodiments, a conservative amino acid substitution can be substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some embodiments, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Exhaustive Matching of the Entire Protein Sequence Database, Science 256:1443-45, hereby incorporated by reference. In some embodiments, the substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

[0071] In some embodiments, residue positions in an immunoglobulin light chain or heavy chain differ by one or more conservative amino acid substitutions. In some embodiments, residue positions in an immunoglobulin light chain or functional fragment thereof (*e.g.,* a fragment that allows expression and secretion from, *e.g.,* a B cell) are not identical to a light chain whose amino acid sequence is listed herein, but differs by one or more conservative amino acid substitutions.

[0072] The phrase "epitope-binding protein" includes a protein having at least one CDR and that is capable of selectively recognizing an epitope, *e.g.,* is capable of binding an epitope with a K_D that is at about one micromolar or lower (*e.g.,* a K_D that is about 1×10^{-6} M, 1×10^{-7}

M, 1 x 10^{-9} M, 1 x 10^{-9} M, 1 x 10^{-10} M, 1 x 10^{-11} M, or about 1 x 10^{-12} M). Therapeutic epitope-binding proteins (*e.g.,* therapeutic antibodies) frequently require a K_D that is in the nanomolar or the picomolar range.

[0073] The phrase "functional fragment" includes fragments of epitope-binding proteins that can be expressed, secreted, and specifically bind to an epitope with a K_D in the micromolar, nanomolar, or picomolar range. Specific recognition includes having a K_D that is at least in the micromolar range, the nanomolar range, or the picomolar range.

[0074] The term "germline" includes reference to an immunoglobulin nucleic acid sequence in a non-somatically mutated cell, *e.g.,* a non-somatically mutated B cell or pre-B cell or hematopoietic cell.

[0075] The phrase "heavy chain," or "immunoglobulin heavy chain" includes an immunoglobulin heavy chain constant region sequence from any organism. Heavy chain variable domains include three heavy chain CDRs and four FR regions, unless otherwise specified. Fragments of heavy chains include CDRs, CDRs and FRs, and combinations thereof. A typical heavy chain has, following the variable domain (from N-terminal to C-terminal), a CH1 domain, a hinge, a CH2 domain, and a CH3 domain. A functional fragment of a heavy chain includes a fragment that is capable of specifically recognizing an epitope (*e.g.,* recognizing the epitope with a K_D in the micromolar, nanomolar, or picomolar range), that is capable of expressing and secreting from a cell, and that comprises at least one CDR.

[0076] The term "identity" when used in connection with sequence, includes identity as determined by a number of different algorithms known in the art that can be used to measure nucleotide and/or amino acid sequence identity. In some embodiments described herein, identities are determined using a ClustalW v. 1.83 (slow) alignment employing an open gap penalty of 10.0, an extend gap penalty of 0.1, and using a Gonnet similarity matrix (MacVector™ 10.0.2, MacVector Inc., 2008). The length of the sequences compared with respect to identity of sequences will depend upon the particular sequences, but in the case of a light chain constant domain, the length should contain sequence of sufficient length to fold into a light chain constant domain that is capable of self-association to form a canonical light chain constant domain, *e.g.,* capable of forming two beta sheets comprising beta strands and capable of interacting with at least one CH1 domain of a human or a mouse. In the case of a CH1 domain, the length of sequence should contain sequence of sufficient length to fold into a CH1 domain that is capable of forming two beta sheets comprising beta strands and capable of interacting with at least one light chain constant domain of a mouse or a human.

[0077] The phrase "immunoglobulin molecule" includes two immunoglobulin heavy chains and two immunoglobulin light chains. The heavy chains may be identical or different, and the light chains may be identical or different.

[0078] The phrase "light chain" includes an immunoglobulin light chain sequence from any organism, and unless otherwise specified includes human κ and λ light chains and a VpreB, as well as surrogate light chains. Light chain variable (VL) domains typically include three light chain CDRs and four framework (FR) regions, unless otherwise specified. Generally, a full-length light chain includes, from amino terminus to carboxyl terminus, a VL domain that includes FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, and a light chain constant domain. Light chains include those, *e.g.,* that do not selectively bind either a first or a second epitope selectively bound by the epitope-binding protein in which they appear. Light chains also include those that bind and recognize, or assist the heavy chain with binding and recognizing, one or more epitopes selectively bound by the epitope-binding protein in which they appear. Common light chains are those derived from a human Vκ1-39Jκ5 gene segment or a human Vκ3-20Jκ1 gene segment, and include somatically mutated (*e.g.,* affinity matured) versions of the same.

[0079] The phrase "micromolar range" is intended to mean 1-999 micromolar; the phrase "nanomolar range" is intended to mean 1-999 nanomolar; the phrase "picomolar range" is intended to mean 1-999 picomolar.

[0080] The phrase "somatically mutated" includes reference to a nucleic acid sequence from a B cell that has undergone class-switching, wherein the nucleic acid sequence of an immunoglobulin variable region (*e.g.,* a heavy chain variable domain or including a heavy chain CDR or FR sequence) in the class-switched B cell is not identical to the nucleic acid sequence in the B cell prior to class-switching, such as, for example, a difference in a CDR or framework nucleic acid sequence between a B cell that has not undergone class-switching and a B cell that has undergone class-switching. "Somatically mutated" includes reference to nucleic acid sequences from affinity-matured B cells that are not identical to corresponding immunoglobulin variable region sequences in B cells that are not affinity-matured (*i.e.,* sequences in the genome of germline cells). The phrase "somatically mutated" also includes reference to an immunoglobulin variable region nucleic acid sequence from a B cell after exposure of the B cell to an epitope of interest, wherein the nucleic acid sequence differs from the corresponding nucleic acid sequence prior to exposure of the B cell to the epitope of interest. The phrase "somatically mutated" refers to sequences from antibodies that have been generated in an animal, *e.g.,* a mouse having human immunoglobulin variable region nucleic acid sequences, in response to an

immunogen challenge, and that result from the selection processes inherently operative in such an animal.

[0081] The term "unrearranged," with reference to a nucleic acid sequence, includes nucleic acid sequences that exist in the germline of an animal cell.

[0082] The phrase "variable domain" includes an amino acid sequence of an immunoglobulin light or heavy chain (modified as desired) that comprises the following amino acid regions, in sequence from N-terminal to C-terminal (unless otherwise indicated): FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

Common Light Chain

[0083] Prior efforts to make useful multispecific epitope-binding proteins, *e.g.,* bispecific antibodies, have been hindered by variety of problems that frequently share a common paradigm: *in vitro* selection or manipulation of sequences to rationally engineer, or to engineer through trial-and-error, a suitable format for pairing a heterodimeric bispecific human immunoglobulin. Unfortunately, most if not all of the *in vitro* engineering approaches provide largely *ad hoc* fixes that are suitable, if at all, for individual molecules. On the other hand, *in vivo* methods for employing complex organisms to select appropriate pairings that are capable of leading to human therapeutics have not been realized.

[0084] Generally, native mouse sequences are frequently not a good source for human therapeutic sequences. For at least that reason, generating mouse heavy chain immunoglobulin variable regions that pair with a common human light chain is of limited practical utility. More *in vitro* engineering efforts would be expended in a trial-and-error process to try to humanize the mouse heavy chain variable sequences while hoping to retain epitope specificity and affinity while maintaining the ability to couple with the common human light chain, with uncertain outcome. At the end of such a process, the final product may maintain some of the specificity and affinity, and associate with the common light chain, but ultimately immunogenicity in a human would likely remain a profound risk.

[0085] Therefore, a suitable mouse for making human therapeutics would include a suitably large repertoire of human heavy chain variable region gene segments in place of endogenous mouse heavy chain variable region gene segments. The human heavy chain variable region gene segments should be able to rearrange and recombine with an endogenous mouse heavy chain constant domain to form a reverse chimeric heavy chain (*i.e.,* a heavy chain comprising a human variable domain and a mouse constant region). The heavy chain should be capable of class switching and somatic hypermutation so that a suitably large repertoire of heavy chain variable domains are available for the mouse to

select one that can associate with the limited repertoire of human light chain variable regions.

[0086] A mouse that selects a common light chain for a plurality of heavy chains has a practical utility. In various embodiments, antibodies that express in a mouse that can only express a common light chain will have heavy chains that can associate and express with an identical or substantially identical light chain. This is particularly useful in making bispecific antibodies. For example, such a mouse can be immunized with a first immunogen to generate a B cell that expresses an antibody that specifically binds a first epitope. The mouse (or a mouse genetically the same) can be immunized with a second immunogen to generate a B cell that expresses an antibody that specifically binds the second epitope. Variable heavy regions can be cloned from the B cells and expresses with the same heavy chain constant region, and the same light chain, and expressed in a cell to make a bispecific antibody, wherein the light chain component of the bispecific antibody has been selected by a mouse to associate and express with the light chain component.

[0087] The inventors have engineered a mouse for generating immunoglobulin light chains that will suitably pair with a rather diverse family of heavy chains, including heavy chains whose variable regions depart from germline sequences, *e.g.,* affinity matured or somatically mutated variable regions. In various embodiments, the mouse is devised to pair human light chain variable domains with human heavy chain variable domains that comprise somatic mutations, thus enabling a route to high affinity binding proteins suitable for use as human therapeutics.

[0088] The genetically engineered mouse, through the long and complex process of antibody selection within an organism, makes biologically appropriate choices in pairing a diverse collection of human heavy chain variable domains with a limited number of human light chain options. In order to achieve this, the mouse is engineered to present a limited number of human light chain variable domain options in conjunction with a wide diversity of human heavy chain variable domain options. Upon challenge with an immunogen, the mouse maximizes the number of solutions in its repertoire to develop an antibody to the immunogen, limited largely or solely by the number or light chain options in its repertoire. In various embodiments, this includes allowing the mouse to achieve suitable and compatible somatic mutations of the light chain variable domain that will nonetheless be compatible with a relatively large variety of human heavy chain variable domains, including in particular somatically mutated human heavy chain variable domains.

[0089] To achieve a limited repertoire of light chain options, the mouse is engineered to render nonfunctional or substantially nonfunctional its ability to make, or rearrange, a native mouse light chain variable domain. This can be achieved, *e.g.,* by deleting the mouse's light

chain variable region gene segments. The endogenous mouse locus can then be modified by an exogenous suitable human light chain variable region gene segment of choice, operably linked to the endogenous mouse light chain constant domain, in a manner such that the exogenous human variable region gene segments can rearrange and recombine with the endogenous mouse light chain constant region gene and form a rearranged reverse chimeric light chain gene (human variable, mouse constant). In various embodiments, the light chain variable region is capable of being somatically mutated. In various embodiments, to maximize ability of the light chain variable region to acquire somatic mutations, the appropriate enhancer(s) is retained in the mouse. For example, in modifying a mouse κ locus to replace endogenous mouse κ variable region gene segments with human κ variable region gene segments, the mouse κ intronic enhancer and mouse κ 3' enhancer are functionally maintained, or undisrupted.

[0090] A genetically engineered mouse is provided that expresses a limited repertoire of reverse chimeric (human variable, mouse constant) light chains associated with a diversity of reverse chimeric (human variable, mouse constant) heavy chains. In various embodiments, the endogenous mouse κ light chain variable region gene segments are deleted and replaced with a single (or two) human light chain variable region gene segments, operably linked to the endogenous mouse κ constant region gene. In embodiments for maximizing somatic hypermutation of the human light chain variable region gene segments, the mouse κ intronic enhancer and the mouse κ 3' enhancer are maintained. In various embodiments, the mouse also comprises a nonfunctional λ light chain locus, or a deletion thereof or a deletion that renders the locus unable to make a λ light chain.

[0091] A genetically engineered mouse is provided that, in various embodiments, comprises a light chain variable region locus lacking an endogenous mouse light chain variable gene segment and comprising a human variable gene segment, in one embodiment a rearranged human V/J sequence, operably linked to a mouse constant region, wherein the locus is capable of undergoing somatic hypermutation, and wherein the locus expresses a light chain comprising the human V/J sequence linked to a mouse constant region. Thus, in various embodiments, the locus comprises a mouse κ 3' enhancer, which is correlated with a normal, or wild-type, level of somatic hypermutation.

[0092] The genetically engineered mouse in various embodiments when immunized with an antigen of interest generates B cells that exhibit a diversity of rearrangements of human immunoglobulin heavy chain variable regions that express and function with one or with two rearranged light chains, including embodiments where the one or two light chains comprise human light chain variable regions that comprise, *e.g.*, 1 to 5 somatic mutations. In various

embodiments, the human light chains so expressed are capable of associating and expressing with any human immunoglobulin heavy chain variable region expressed in the mouse.

Epitope-binding Proteins Binding More Than One Epitope

[0093] The compositions and methods of described herein can be used to make binding proteins that bind more than one epitope with high affinity, *e.g.,* bispecific antibodies. Advantages of the invention include the ability to select suitably high binding (*e.g.,* affinity matured) heavy chain immunoglobulin chains each of which will associate with a single light chain.

[0094] Synthesis and expression of bispecific binding proteins has been problematic, in part due to issues associated with identifying a suitable light chain that can associate and express with two different heavy chains, and in part due to isolation issues. The methods and compositions described herein allow for a genetically modified mouse to select, through otherwise natural processes, a suitable light chain that can associate and express with more than one heavy chain, including heavy chains that are somatically mutated (*e.g.,* affinity matured). Human VL and VH sequences from suitable B cells of immunized mice as described herein that express affinity matured antibodies having reverse chimeric heavy chains (i.e., human variable and mouse constant) can be identified and cloned in frame in an expression vector with a suitable human constant region gene sequence (*e.g.,* a human IgG1). Two such constructs can be prepared, wherein each construct encodes a human heavy chain variable domain that binds a different epitope. One of the human VLs (*e.g.,* human Vκ1-39Jκ5 or human Vκ3-20Jκ1), in germline sequence or from a B cell wherein the sequence has been somatically mutated, can be fused in frame to a suitable human constant region gene (*e.g.,* a human κ constant gene). These three fully-human heavy and light constructs can be placed in a suitable cell for expression. The cell will express two major species: a homodimeric heavy chain with the identical light chain, and a heterodimeric heavy chain with the identical light chain. To allow for a facile separation of these major species, one of the heavy chains is modified to omit a Protein A-binding determinant, resulting in a differential affinity of a homodimeric binding protein from a heterodimeric binding protein. Compositions and methods that address this issue are described in USSN 12/832,838, filed 25 June 20010, entitled "Readily Isolated Bispecific Antibodies with Native Immunoglobulin Format," published as US 2010/0331527A1, hereby incorporated by reference.

[0095] In one aspect, an epitope-binding protein as described herein is provided, wherein human VL and VH sequences are derived from mice described herein that have been immunized with an antigen comprising an epitope of interest.

[0096] In one embodiment, an epitope-binding protein is provided that comprises a first and a second polypeptide, the first polypeptide comprising, from N-terminal to C-terminal, a first epitope-binding region that selectively binds a first epitope, followed by a constant region that comprises a first CH3 region of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof; and, a second polypeptide comprising, from N-terminal to C-terminal, a second epitope-binding region that selectively binds a second epitope, followed by a constant region that comprises a second CH3 region of a human IgG selected from IgG1, IgG2, IgG4, and a combination thereof, wherein the second CH3 region comprises a modification that reduces or eliminates binding of the second CH3 domain to protein A.

[0097] In one embodiment, the second CH3 region comprises an H95R modification (by IMGT exon numbering; H435R by EU numbering). In another embodiment, the second CH3 region further comprises a Y96F modification (IMGT; Y436F by EU).

[0098] In one embodiment, the second CH3 region is from a modified human IgG1, and further comprises a modification selected from the group consisting of D16E, L18M, N44S, K52N, V57M, and V82I (IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU).

[0099] In one embodiment, the second CH3 region is from a modified human IgG2, and further comprises a modification selected from the group consisting of N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU).

[00100] In one embodiment, the second CH3 region is from a modified human IgG4, and further comprises a modification selected from the group consisting of Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU).

[00101] One method for making an epitope-binding protein that binds more than one epitope is to immunize a first mouse in accordance with the invention with an antigen that comprises a first epitope of interest, wherein the mouse comprises an endogenous immunoglobulin light chain variable region locus that does not contain an endogenous mouse VL that is capable of rearranging and forming a light chain, wherein at the endogenous mouse immunglobulin light chain variable region locus is a single human VL gene segment operably linked to the mouse endogenous light chain constant region gene, and the human VL gene segment is selected from a human Vκ1-39Jκ5 and a human Vκ3-20Jκ1, and the endogenous mouse VH gene segments have been replaced in whole or in part with human VH gene segments, such that immunoglobulin heavy chains made by the mouse are solely or substantially heavy chains that comprise human variable domains and

mouse constant domains. When immunized, such a mouse will make a reverse chimeric antibody, comprising only one of two human light chain variable domains (*e.g.,* one of human Vκ1-39Jκ5 or human Vκ3-20Jκ1). Once a B cell is identified that encodes a VH that binds the epitope of interest, the nucleotide sequence of the VH (and, optionally, the VL) can be retrieved (*e.g.,* by PCR) and cloned into an expression construct in frame with a suitable human immunoglobulin constant domain. This process can be repeated to identify a second VH domain that binds a second epitope, and a second VH gene sequence can be retrieved and cloned into an expression vector in frame to a second suitable immunoglobulin constant domain. The first and the second immunoglobulin constant domains can the same or different isotype, and one of the immunoglobulin constant domains (but not the other) can be modified as described herein or in US 2010/0331527A1, and epitope-binding protein can be expressed in a suitable cell and isolated based on its differential affinity for Protein A as compared to a homodimeric epitope-binding protein, *e.g.,* as described in US 2010/0331527A1.

[00102] In one embodiment, a method for making a bispecific epitope-binding protein is provided, comprising identifying a first affinity-matured (*e.g.,* comprising one or more somatic hypermutations) human VH nucleotide sequence (VH1) from a mouse as described herein, identifying a second affinity-matured (*e.g.,* comprising one or more somatic hypermutations) human VH nucleotide sequence (VH2) from a mouse as described herein, cloning VH1 in frame with a human heavy chain lacking a Protein A-determinant modification as described in US 2010/0331527A1 for form heavy chain 1 (HC1), cloning VH2 in frame with a human heavy chain comprising a Protein A-determinant as described in US 2010/0331527A1 to form heavy chain 2 (HC2), introducing an expression vector comprising HC1 and the same or a different expression vector comprising HC2 into a cell, wherein the cell also expresses a human immunoglobulin light chain that comprises a human Vκ1-39/human Jκ5 or a human Vκ3-20/human Jκ1 fused to a human light chain constant domain, allowing the cell to express a bispecific epitope-binding protein comprising a VH domain encoded by VH1 and a VH domain encoded by VH2, and isolating the bispecific epitope-binding protein based on its differential ability to bind Protein A as compared with a monospecific homodimeric epitope-binding protein. In a specific embodiment, HC1 is an IgG1, and HC2 is an IgG1 that comprises the modification H95R (IMGT; H435R by EU) and further comprises the modification Y96F (IMGT; Y436F by EU). In one embodiment, the VH domain encoded by VH1, the VH domain encoded by VH2, or both, are somatically mutated.

Human VH Genes That Express with a Common Human VL

[00103] A variety of human variable regions from affinity-matured antibodies raised against four different antigens were expressed with either their cognate light chain, or at least one of a human light chain selected from human Vκ1-39Jκ5, human Vκ3-20Jκ1, or human VpreBJλ5 (see Example 1). For antibodies to each of the antigens, somatically mutated high affinity heavy chains from different gene families paired successfully with rearranged human germline Vκ1-39Jκ5 and Vκ3-20Jκ1 regions and were secreted from cells expressing the heavy and light chains. For Vκ1-39Jκ5 and Vκ3-20Jκ1, VH domains derived from the following human VH families expressed favorably: 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, and 6-1. Thus, a mouse that is engineered to express a limited repertoire of human VL domains from one or both of Vκ1-39Jκ5 and Vκ3-20Jκ1 will generate a diverse population of somatically mutated human VH domains from a VH locus modified to replace mouse VH gene segments with human VH gene segments.

[00104] Mice genetically engineered to express reverse chimeric (human variable, mouse constant) immunoglobulin heavy chains associated with a single rearranged light chain (*e.g.*, a Vκ1-39/J or a Vκ3-20/J), when immunized with an antigen of interest, generated B cells that comprised a diversity of human V segment rearrangements and expressed a diversity of high-affinity antigen-specific antibodies with diverse properties with respect to their ability to block binding of the antigen to its ligand, and with respect to their ability to bind variants of the antigen (see Examples 5 through 10).

[00105] Thus, the mice and methods described herein are useful in making and selecting human immunoglobulin heavy chain variable domains, including somatically mutated human heavy chain variable domains, that result from a diversity of rearrangements, that exhibit a wide variety of affinities (including exhibiting a K_D of about a nanomolar or less), a wide variety of specificities (including binding to different epitopes of the same antigen), and that associate and express with the same or substantially the same human immunoglobulin light chain variable region.

[00106] The following examples are provided so as to describe to those of ordinary skill in the art how to make and use methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.,* amounts, temperature, *etc.*) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is indicated in Celsius, and pressure is at or near atmospheric.

EXAMPLES

Example 1. Identification of human heavy chain variable regions that associate with selected human light chain variable regions

[00107] An *in vitro* expression system was constructed to determine if a single rearranged human germline light chain could be co-expressed with human heavy chains from antigen specific human antibodies.

[00108] Methods for generating human antibodies in genetically modified mice are known (see *e.g.,* US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®). The VELOCIMMUNE® technology involves generation of a genetically modified mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibodies produced from a VELOCIMMUNE® mouse are fully human. Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As described below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, *etc.* The mouse constant regions are replaced with a desired human constant region to generate a fully human antibody containing a non-IgM isotype, for example, wild-type or modified IgG1, IgG2, IgG3 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[00109] A VELOCIMMUNE® mouse was immunized with a growth factor that promotes angiogenesis (Antigen C) and antigen-specific human antibodies were isolated and sequenced for V gene usage using standard techniques recognized in the art. Selected antibodies were cloned onto human heavy and light chain constant regions and 69 heavy chains were selected for pairing with one of three human light chains: (1) the cognate κ light chain linked to a human κ constant region, (2) a rearranged human germline Vκ1-39Jκ5 linked to a human κ constant region, or (3) a rearranged human germline Vκ3-20Jκ1 linked to a human κ constant region. Each heavy chain and light chain pair were co-transfected in CHO-K1 cells using standard techniques. Presence of antibody in the supernatant was detected by anti-human IgG in an ELISA assay. Antibody titer (ng/ml) was determined for each heavy chain/light chain pair and titers with the different rearranged germline light chains were compared to the titers obtained with the parental antibody molecule (*i.e.*, heavy chain paired with cognate light chain) and percent of native titer was calculated (Table 1).

V_H: Heavy chain variable gene. ND: no expression detected under current experimental conditions.

[00110]

Table 1					
V_H	Antibody Titer (ng/ml)			Percent of Native Titer	
	Cognate LC	Vκ1-39Jκ5	Vκ3-20Jκ1	Vκ1-39Jκ5	Vκ3-20Jκ1
3-15	63	23	11	36.2	17.5
1-2	103	53	ND	51.1	-
3-23	83	60	23	72.0	27.5
3-33	15	77	ND	499.4	-
4-31	22	69	17	309.4	76.7
3-7	53	35	28	65.2	53.1
-	22	32	19	148.8	89.3
1-24	3	13	ND	455.2	-
3-33	1	47	ND	5266.7	-
3-33	58	37	ND	63.1	-
-	110	67	18	60.6	16.5
3-23	127	123	21	96.5	16.3
3-33	28	16	2	57.7	7.1
3-23	32	50	38	157.1	119.4
-	18	45	18	254.3	101.7
3-9	1	30	23	2508.3	1900.0
3-11	12	26	6	225.9	48.3
1-8	16	ND	13	-	81.8
3-33	54	81	10	150.7	19.1
-	34	9	ND	25.9	-
3-20	7	14	54	203.0	809.0
3-33	19	38	ND	200.5	-
3-11	48	ND	203	-	423.6
-	11	23	8	212.7	74.5
3-33	168	138	182	82.0	108.2
3-20	117	67	100	57.5	86.1
3-23	86	61	132	70.7	154.1
3-33	20	12	33	60.9	165.3
4-31	69	92	52	133.8	75.0
3-23	87	78	62	89.5	71.2
1-2	31	82	51	263.0	164.6
3-23	53	93	151	175.4	285.4

-	11	8	17	75.7	151.4
3-33	114	36	27	31.6	23.4
3-15	73	39	44	53.7	59.6
3-33	1	34	16	5600.0	2683.3
3-9	58	112	57	192.9	97.6
3-33	67	20	105	30.1	157.0
3-33	34	21	24	62.7	70.4
3-20	10	49	91	478.4	888.2
3-33	66	32	25	48.6	38.2
3-23	17	59	56	342.7	329.8
-	58	108	19	184.4	32.9
-	68	54	20	79.4	29.9
3-33	42	35	32	83.3	75.4
-	29	19	13	67.1	43.9
3-9	24	34	29	137.3	118.4
3-30/33	17	33	7	195.2	43.1
3-7	25	70	74	284.6	301.6
3-33	87	127	ND	145.1	-
6-1	28	56	ND	201.8	-
3-33	56	39	20	69.9	36.1
3-33	10	53	1	520.6	6.9
3-33	20	67	10	337.2	52.3
3-33	11	36	18	316.8	158.4
3-23	12	42	32	356.8	272.9
3-33	66	95	15	143.6	22.5
3-15	55	68	ND	123.1	-
-	32	68	3	210.9	10.6
1-8	28	48	ND	170.9	-
3-33	124	192	21	154.3	17.0
3-33	0	113	ND	56550.0	-
3-33	10	157	1	1505.8	12.5
3-33	6	86	15	1385.5	243.5
3-23	70	115	22	163.5	31.0
3-7	71	117	21	164.6	29.6
3-33	82	100	47	122.7	57.1
3-7	124	161	41	130.0	33.5

[00111] In a similar experiment, VELOCIMMUNE® mice were immunized with several different antigens and selected heavy chains of antigen specific human antibodies were tested for their ability to pair with different rearranged human germline light chains (as

described above). The antigens used in this experiment included an enzyme involved in cholesterol homeostasis (Antigen A), a serum hormone involved in regulating glucose homeostasis (Antigen B), a growth factor that promotes angiogenesis (Antigen C) and a cell-surface receptor (Antigen D). Antigen specific antibodies were isolated from mice of each immunization group and the heavy chain and light chain variable regions were cloned and sequenced. From the sequence of the heavy and light chains, V gene usage was determined and selected heavy chains were paired with either their cognate light chain or a rearranged human germline Vκ1-39Jκ5 region. Each heavy/light chain pair was co-transfected in CHO-K1 cells and the presence of antibody in the supernatant was detected by anti-human IgG in an ELISA assay. Antibody titer (μg/ml) was determined for each heavy chain/light chain pairing and titers with the different rearranged human germline light chains were compared to the titers obtained with the parental antibody molecule (*i.e.*, heavy chain paired with cognate light chain) and percent of native titer was calculated (Table 2). V_H: Heavy chain variable gene. Vκ: κ light chain variable gene. ND: no expression detected under current experimental conditions.

[00112]

Table 2

Antigen	Antibody	V_H	Vκ	Titer (μg/ml)			Percent of Native Titer
				V_H Alone	V_H + Vκ	V_H + Vκ1-39Jκ5	
A	320	1-18	2-30	0.3	3.1	2.0	66
	321	2-5	2-28	0.4	0.4	1.9	448
	334	2-5	2-28	0.4	2.7	2.0	73
	313	3-13	3-15	0.5	0.7	4.5	670
	316	3-23	4-1	0.3	0.2	4.1	2174
	315	3-30	4-1	0.3	0.2	3.2	1327
	318	4-59	1-17	0.3	4.6	4.0	86
B	257	3-13	1-5	0.4	3.1	3.2	104
	283	3-13	1-5	0.4	5.4	3.7	69
	637	3-13	1-5	0.4	4.3	3.0	70
	638	3-13	1-5	0.4	4.1	3.3	82
	624	3-23	1-17	0.3	5.0	3.9	79
	284	3-30	1-17	0.3	4.6	3.4	75
	653	3-33	1-17	0.3	4.3	0.3	7
	268	4-34	1-27	0.3	5.5	3.8	69
	633	4-34	1-27	0.6	6.9	3.0	44
C	730	3-7	1-5	0.3	1.1	2.8	249
	728	3-7	1-5	0.3	2.0	3.2	157

	691	3-9	3-20	0.3	2.8	3.1	109
	749	3-33	3-15	0.3	3.8	2.3	62
	750	3-33	1-16	0.3	3.0	2.8	92
	724	3-33	1-17	0.3	2.3	3.4	151
	706	3-33	1-16	0.3	3.6	3.0	84
	744	1-18	1-12	0.4	5.1	3.0	59
	696	3-11	1-16	0.4	3.0	2.9	97
	685	3-13	3-20	0.3	0.5	3.4	734
	732	3-15	1-17	0.3	4.5	3.2	72
	694	3-15	1-5	0.4	5.2	2.9	55
	743	3-23	1-12	0.3	3.2	0.3	10
	742	3-23	2-28	0.4	4.2	3.1	74
	693	3-23	1-12	0.5	4.2	4.0	94
D	136	3-23	2-28	0.4	5.0	2.7	55
	155	3-30	1-16	0.4	1.0	2.2	221
	163	3-30	1-16	0.3	0.6	3.0	506
	171	3-30	1-16	0.3	1.0	2.8	295
	145	3-43	1-5	0.4	4.4	2.9	65
	49	3-48	3-11	0.3	1.7	2.6	155
	51	3-48	1-39	0.1	1.9	0.1	4
	159	3-7	6-21	0.4	3.9	3.6	92
	169	3-7	6-21	0.3	1.3	3.1	235
	134	3-9	1-5	0.4	5.0	2.9	58
	141	4-31	1-33	2.4	4.2	2.6	63
	142	4-31	1-33	0.4	4.2	2.8	67

[00113] The results obtained from these experiments demonstrate that somatically mutated, high affinity heavy chains from different gene families are able to pair with rearranged human germline Vκ1-39Jκ5 and Vκ3-20Jκ1 regions and be secreted from the cell as a normal antibody molecule. As shown in Table 1, antibody titer was increased for about 61% (42 of 69) heavy chains when paired with the rearranged human Vκ1-39Jκ5 light chain and about 29% (20 of 69) heavy chains when paired with the rearranged human Vκ3-20Jκ1 light chain as compared to the cognate light chain of the parental antibody. For about 20% (14 of 69) of the heavy chains, both rearranged human germline light chains conferred an increase in expression as compared to the cognate light chain of the parental antibody. As shown in Table 2, the rearranged human germline Vκ1-39Jκ5 region conferred an increase in expression of several heavy chains specific for a range of different classes of antigens as compared to the cognate light chain for the parental antibodies. Antibody titer was increased by more than two-fold for about 35% (15/43) of the heavy chains as

compared to the cognate light chain of the parental antibodies. For two heavy chains (315 and 316), the increase was greater than ten-fold as compared to the parental antibody. Within all the heavy chains that showed increase expression relative to the cognate light chain of the parental antibody, family three (V_H3) heavy chains are over represented in comparison to other heavy chain variable region gene families. This demonstrates a favorable relationship of human V_H3 heavy chains to pair with rearranged human germline Vκ1-39Jκ5 and Vκ3-20Jκ1 light chains.

Example 2. Generation of a Rearranged Human Germline Light Chain Locus

[00114] Various rearranged human germline light chain targeting vectors were made using VELOCIGENE® technology (see, *e.g.,* US Pat. No. 6,586,251 and Valenzuela *et al.* (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, Nature Biotech. 21(6):652-659) to modify mouse genomic Bacterial Artificial Chromosome (BAC) clones 302g12 and 254m04 (Invitrogen). Using these two BAC clones, genomic constructs were engineered to contain a single rearranged human germline light chain region and inserted into an endogenous κ light chain locus that was previously modified to delete the endogenous κ variable and joining gene segments.

A. Construction of Rearranged Human Germline Light Chain Targeting Vectors

[00115] Three different rearranged human germline light chain regions were made using standard molecular biology techniques recognized in the art. The human variable gene segments used for constructing these three regions included rearranged human Vκ1-39Jκ5 sequence, a rearranged human Vκ3-20Jκ1 sequence and a rearranged human VpreBJλ5 sequence.

[00116] A DNA segment containing exon 1 (encoding the leader peptide) and intron 1 of the mouse Vκ3-7 gene was made by de novo DNA synthesis (Integrated DNA Technologies). Part of the 5' untranslated region up to a naturally occurring BlpI restriction enzyme site was included. Exons of human Vκ1-39 and Vκ3-20 genes were PCR amplified from human genomic BAC libraries. The forward primers had a 5' extension containing the splice acceptor site of intron 1 of the mouse Vκ3-7 gene. The reverse primer used for PCR of the human Vκ1-39 sequence included an extension encoding human Jκ5, whereas the reverse primer used for PCR of the human Vκ3-20 sequence included an extension encoding human Jκ1. The human VpreBJλ5 sequence was made by de novo DNA synthesis (Integrated DNA Technologies). A portion of the human Jκ-Cκ intron including the splice donor site was PCR amplified from plasmid pBS-296-HA18-PIScel. The forward PCR

primer included an extension encoding part of either a human Jκ5, Jκ1, or Jλ5 sequence. The reverse primer included a PI-SceI site, which was previously engineered into the intron.

[00117] The mouse Vκ3-7 exon1/intron 1, human variable light chain exons, and human Jκ-Cκ intron fragments were sewn together by overlap extension PCR, digested with BlpI and PI-SceI, and ligated into plasmid pBS-296-HA18-PISceI, which contained the promoter from the human Vκ3-15 variable gene segment. A loxed hygromycin cassette within plasmid pBS-296-HA18-PISceI was replaced with a FRTed hygromycin cassette flanked by NotI and AscI sites. The NotI/PI-SceI fragment of this plasmid was ligated into modified mouse BAC 254m04, which contained part of the mouse Jκ-Cκ intron, the mouse Cκ exon, and about 75 kb of genomic sequence downstream of the mouse κ locus which provided a 3' homology arm for homologous recombination in mouse ES cells. The NotI/AscI fragment of this BAC was then ligated into modified mouse BAC 302g12, which contained a FRTed neomycin cassette and about 23 kb of genomic sequence upstream of the endogenous κ locus for homologous recombination in mouse ES cells.

B. Rearranged Human Germline Vκ1-39Jκ5 Targeting Vector (Figure 1)

[00118] Restriction enzyme sites were introduced at the 5' and 3' ends of an engineered light chain insert for cloning into a targeting vector: an AscI site at the 5' end and a PI-SceI site at the 3' end. Within the 5' AscI site and the 3' PI-SceI site the targeting construct from 5' to 3' included a 5' homology arm containing sequence 5' to the endogenous mouse κ light chain locus obtained from mouse BAC clone 302g12, a FRTed neomycin resistance gene, an genomic sequence including the human Vκ3-15 promoter, a leader sequence of the mouse Vκ3-7 variable gene segment, a intron sequence of the mouse Vκ3-7 variable gene segment, an open reading frame of a rearranged human germline Vκ1-39Jκ5 region, a genomic sequence containing a portion of the human Jκ-Cκ intron, and a 3' homology arm containing sequence 3' of the endogenous mouse Jκ5 gene segment obtained from mouse BAC clone 254m04 (Figure 1, middle). Genes and/or sequences upstream of the endogenous mouse κ light chain locus and downstream of the most 3' Jκ gene segment (*e.g.*, the endogenous 3' enhancer) were unmodified by the targeting construct (see Figure 1). The sequence of the engineered human Vκ1-39Jκ5 locus is shown in SEQ ID NO:1.

[00119] Targeted insertion of the rearranged human germline Vκ1-39Jκ5 region into BAC DNA was confirmed by polymerase chain reaction (PCR) using primers located at sequences within the rearranged human germline light chain region. Briefly, the intron sequence 3' to the mouse Vκ3-7 leader sequence was confirmed with primers ULC-m1F (AGGTGAGGGT ACAGATAAGT GTTATGAG; SEQ ID NO:2) and ULC-m1R

(TGACAAATGC CCTAATTATA GTGATCA; SEQ ID NO:3). The open reading frame of the rearranged human germline Vκ1-39Jκ5 region was confirmed with primers 1633-h2F (GGGCAAGTCA GAGCATTAGC A; SEQ ID NO:4) and 1633-h2R (TGCAAACTGG ATGCAGCATA G; SEQ ID NO:5). The neomycin cassette was confirmed with primers neoF (GGTGGAGAGG CTATTCGGC; SEQ ID NO:6) and neoR (GAACACGGCG GCATCAG; SEQ ID NO:7). Targeted BAC DNA was then used to electroporate mouse ES cells to created modified ES cells for generating chimeric mice that express a rearranged human germline Vκ1-39Jκ5 region.

[00120] Positive ES cell clones were confirmed by TAQMAN™ screening and karyotyping using probes specific for the engineered Vκ1-39Jκ5 light chain region inserted into the endogenous locus. Briefly, probe neoP (TGGGCACAAC AGACAATCGG CTG; SEQ ID NO:8) which binds within the neomycin marker gene, probe ULC-m1P (CCATTATGAT GCTCCATGCC TCTCTGTTC; SEQ ID NO:9) which binds within the intron sequence 3' to the mouse Vκ3-7 leader sequence, and probe 1633h2P (ATCAGCAGAA ACCAGGGAAA GCCCCT; SEQ ID NO:10) which binds within the rearranged human germline Vκ1-39Jκ5 open reading frame. Positive ES cell clones were then used to implant female mice to give rise to a litter of pups expressing the germline Vκ1-39Jκ5 light chain region.

[00121] Alternatively, ES cells bearing the rearranged human germline Vκ1-39Jκ5 light chain region are transfected with a constuct that expresses FLP in order to remove the FRTed neomycin cassette introduced by the targeting construct. Optionally, the neomycin cassette is removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice

C. Rearranged Human Germline Vκ3-20Jκ1 Targeting Vector (Figure 2)

[00122] In a similar fashion, an engineered light chain locus expressing a rearranged human germline Vκ3-20Jκ1 region was made using a targeting construct including, from 5' to 3', a 5' homology arm containing sequence 5' to the endogenous mouse κ light chain locus obtained from mouse BAC clone 302g12, a FRTed neomycin resistance gene, a genomic sequence including the human Vκ3-15 promoter, a leader sequence of the mouse Vκ3-7 variable gene segment, an intron sequence of the mouse Vκ3-7 variable gene segment, an open reading frame of a rearranged human germline Vκ3-20Jκ1 region, a genomic sequence containing a portion of the human Jκ-Cκ intron, and a 3' homology arm containing sequence 3' of the endogenous mouse Jκ5 gene segment obtained from mouse BAC clone 254m04 (Figure 2, middle). The sequence of the engineered human Vκ3-20Jκ1 locus is shown in SEQ ID NO:11.

[00123] Targeted insertion of the rearranged human germline Vκ3-20Jκ1 region into BAC DNA was confirmed by polymerase chain reaction (PCR) using primers located at sequences within the rearranged human germline Vκ3-20Jκ1 light chain region. Briefly, the intron sequence 3' to the mouse Vκ3-7 leader sequence was confirmed with primers ULC-m1F (SEQ ID NO:2) and ULC-m1R (SEQ ID NO:3). The open reading frame of the rearranged human germline Vκ3-20Jκ1 region was confirmed with primers 1635-h2F (TCCAGGCACC CTGTCTTTG; SEQ ID NO:12) and 1635-h2R (AAGTAGCTGC TGCTAACACT CTGACT; SEQ ID NO:13). The neomycin cassette was confirmed with primers neoF (SEQ ID NO:6) and neoR (SEQ ID NO:7). Targeted BAC DNA was then used to electroporate mouse ES cells to created modified ES cells for generating chimeric mice that express the rearranged human germline Vκ3-20Jκ1 light chain.

[00124] Positive ES cell clones were confirmed by Taqman™ screening and karyotyping using probes specific for the engineered Vκ3-20Jκ1 light chain region inserted into the endogenous κ light chain locus. Briefly, probe neoP (SEQ ID NO:8) which binds within the neomycin marker gene, probe ULC-m1P (SEQ ID NO:9) which binds within the mouse Vκ3-7 leader sequence, and probe 1635h2P (AAAGAGCCAC CCTCTCCTGC AGGG; SEQ ID NO:14) which binds within the human Vκ3-20Jκ1 open reading frame. Positive ES cell clones were then used to implant female mice. A litter of pups expressing the human germline Vκ3-20Jκ1 light chain region.

[00125] Alternatively, ES cells bearing human germline Vκ3-20Jκ1 light chain region can be transfected with a constuct that expresses FLP in oder to remove the FRTed neomycin cassette introduced by the targeting consruct. Optionally, the neomycin cassette may be removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice.

D. Rearranged Human Germline VpreBJλ5 Targeting Vector (Figure 3)

[00126] In a similar fashion, an engineered light chain locus expressing a rearranged human germline VpreBJλ5 region was made using a targeting construct including, from 5' to 3', a 5' homology arm containing sequence 5' to the endogenous mouse κ light chain locus obtained from mouse BAC clone 302g12, a FRTed neomycin resistance gene, an genomic sequence including the human Vκ3-15 promoter, a leader sequence of the mouse Vκ3-7 variable gene segment, an intron sequence of the mouse Vκ3-7 variable gene segment, an open reading frame of a rearranged human germline VpreBJλ5 region, a genomic sequence containing a portion of the human Jκ-Cκ intron, and a 3' homology arm containing sequence 3' of the endogenous mouse Jκ5 gene segment obtained from mouse BAC clone 254m04

(Figure 3, middle). The sequence of the engineered human VpreBJλ5 locus is shown in SEQ ID NO:15.

[00127] Targeted insertion of the rearranged human germline VpreBJλ5 region into BAC DNA was confirmed by polymerase chain reaction (PCR) using primers located at sequences within the rearranged human germline VpreBJλ5 region light chain region. Briefly, the intron sequence 3' to the mouse Vκ3-7 leader sequence was confirmed with primers ULC-m1F (SEQ ID NO:2) and ULC-m1R (SEQ ID NO:3). The open reading frame of the rearranged human germline VpreBJλ5 region was confirmed with primers 1616-h1F (TGTCCTCGGC CCTTGGA; SEQ ID NO:16) and 1616-h1R (CCGATGTCAT GGTCGTTCCT; SEQ ID NO:17). The neomycin cassette was confirmed with primers neoF (SEQ ID NO:6) and neoR (SEQ ID NO:7). Targeted BAC DNA was then used to electroporate mouse ES cells to created modified ES cells for generating chimeric mice that express the rearranged human germline VpreBJλ5 light chain.

[00128] Positive ES cell clones are confirmed by TAQMAN™ screening and karyotyping using probes specific for the engineered VpreBJλ5 light chain region inserted into the endogenous κ light chain locus. Briefly, probe neoP (SEQ ID NO:8) which binds within the neomycin marker gene, probe ULC-m1P (SEQ ID NO:9) which binds within the mouse IgVκ3-7 leader sequence, and probe 1616h1P (ACAATCCGCC TCACCTGCAC CCT; SEQ ID NO:18) which binds within the human VpreBJλ5 open reading frame. Positive ES cell clones are then used to implant female mice to give rise to a litter of pups expressing a germline light chain region.

[00129] Alternatively, ES cells bearing the rearranged human germline VpreBJλ5 light chain region are transfected with a construct that expresses FLP in order to remove the FRTed neomycin cassette introduced by the targeting consruct. Optionally, the neomycin cassette is removed by breeding to mice that express FLP recombinase (*e.g.,* US 6,774,279). Optionally, the neomycin cassette is retained in the mice.

Example 3. Generation of Mice expressing a single rearranged human germline light chain

[00130] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE® method (see, *e.g.,* US Pat. No. 7,294,754 and Poueymirou *et al.* (2007) F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99. VELOCIMICE® independently bearing an engineered human germline Vκ1-39Jκ5 light chain region, a Vκ3-20Jκ1 light chain region or a VpreBJλ5 light chain region are identified by genotyping using a modification of allele assay (Valenzuela *et*

al., supra) that detects the presence of the unique rearranged human germline light chain region.

[00131] Pups are genotyped and a pup heterozygous for the unique rearranged human germline light chain region are selected for characterizing expression of the rearranged human germline light chain region.

Example 4. Breeding of Mice expressing a single rearranged human germline light chain

A. Endogenous Igλ Knockout (KO).

[00132] To optimize the usage of the engineered light chain locus, mice bearing one of the rearranged human germline light chain regions are bred to another mouse containing a deletion in the endogenous λ light chain locus. In this manner, the progeny obtained will express, as their only light chain, the rearranged human germline light chain region as described in Example 2. Breeding is performed by standard techniques recognized in the art and, alternatively, by a commercial breeder (*e.g.,* The Jackson Laboratory). Mouse strains bearing an engineered light chain locus and a deletion of the endogenous λ light chain locus are screened for presence of the unique light chain region and absence of endogenous mouse λ light chains.

B. Humanized Endogenous Heavy Chain Locus.

[00133] Mice bearing an engineered human germline light chain locus are bred with mice that contain a replacement of the endogenous mouse heavy chain variable gene locus with the human heavy chain variable gene locus (see US 6,596,541; the VELOCIMMUNE® mouse, Regeneron Pharmaceuticals, Inc.). The VELOCIMMUNE® mouse comprises a genome comprising human heavy chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces antibodies comprising a human heavy chain variable region and a mouse heavy chain constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy chains of the antibodies is isolated and operably linked to DNA encoding the human heavy chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human heavy chain of the antibody.

[00134] Mice bearing a replacement of the endogenous mouse VH locus with the human VH locus and a single rearranged human germline VL region at the endogenous κ light chain locus are obtained. Reverse chimeric antibodies containing somatically mutated heavy chains (human VH and mouse CH) with a single human light chain (human VL and mouse CL) are obtained upon immunization with an antigen of interest. VH and VL

nucleotide sequences of B cells expressing the antibodies are identified and fully human antibodies are made by fusion the VH and VL nucleotide sequences to human CH and CL nucleotide sequences in a suitable expression system.

Example 5. Generation of Antibodies from Mice Expressing Human Heavy Chains and a Rearranged Human Germline Light Chain Region

[00135] After breeding mice that contain the engineered human light chain region to various desired strains containing modifications and deletions of other endogenous Ig loci (as described in Example 4), selected mice can be immunized with an antigen of interest.

[00136] Generally, a VELOCIMMUNE® mouse containing one of the single rearranged human germline light chain regions is challenged with an antigen, and lymphatic cells (such as B-cells) are recovered from serum of the animals. The lymphatic cells are fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies containing human heavy chain variables and a rearranged human germline light chains which are specific to the antigen used for immunization. DNA encoding the variable regions of the heavy chains and the light chain are isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Due to the presence of the endogenous mouse sequences and any additional cis-acting elements present in the endogenous locus, the single light chain of each antibody may be somatically mutated. This adds additional diversity to the antigen-specific repertoire comprising a single light chain and diverse heavy chain sequences. The resulting cloned antibody sequences are subsequently expressed in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains are identified directly from antigen-specific lymphocytes.

[00137] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As described above, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, *etc.* The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody containing a somatically mutated human heavy chain and a single light chain derived from a rearranged human germline light chain region of the invention. Suitable human constant regions include, for example wild-type or modified IgG1 or IgG4.

[00138] Separate cohorts of VELOCIMMUNE® mice containing a replacement of the endogenous mouse heavy chain locus with human V, D, and J gene segments and a replacement of the endogenous mouse κ light chain locus with either the engineered germline Vκ1-39Jκ5 human light chain region or the engineered germline Vκ3-20Jκ1 human

light chain region (described above) were immunized with a human cell-surface receptor protein (Antigen E). Antigen E is administered directly onto the hind footpad of mice with six consecutive injections every 3-4 days. Two to three micrograms of Antigen E are mixed with 10 μg of CpG oligonucleotide (Cat # tlrl-modn - ODN1826 oligonucleotide ; InVivogen, San Diego, CA) and 25 μg of Adju-Phos (Aluminum phosphate gel adjuvant, Cat# H-71639-250; Brenntag Biosector, Frederikssund, Denmark) prior to injection. A total of six injections are given prior to the final antigen recall, which is given 3-5 days prior to sacrifice. Bleeds after the 4th and 6th injection are collected and the antibody immune response is monitored by a standard antigen-specific immunoassay.

[00139] When a desired immune response is achieved splenocytes are harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines are screened and selected to identify cell lines that produce Antigen E-specific common light chain antibodies. Using this technique several anti-Antigen E-specific common light chain antibodies (*i.e.*, antibodies possessing human heavy chain variable domains, the same human light chain variable domain, and mouse constant domains) are obtained.

[00140] Alternatively, anti-Antigen E common light chain antibodies are isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-Antigen E common light chain antibodies (*i.e.,* antibodies possessing human heavy chain variable domains, either an engineered human Vκ1-39Jκ5 light chain or an engineered human Vκ3-20Jκ1 light chain region, and human constant domains) were obtained.

[00141] The biological properties of the exemplary anti-Antigen E common light chain antibodies generated in accordance with the methods of this Example are described in detail in the sections set forth below.

Example 6. Heavy Chain Gene Segment Usage in Antigen-Specific Common Light Chain Antibodies

[00142] To analyze the structure of the human anti-Antigen E common light chain antibodies produced, nucleic acids encoding heavy chain antibody variable regions were cloned and sequenced. From the nucleic acid sequences and predicted amino acid sequences of the antibodies, gene usage was identified for the heavy chain variable region (HCVR) of selected common light chain antibodies obtained from immunized VELOCIMMUNE® mice containing either the engineered human Vκ1-39Jκ5 light chain or engineered human Vκ3-20Jκ1 light chain region. Results are shown in Tables 3 and 4,

which demonstrate that mice according to the invention generate antigen-specific common light chain antibodies from a variety of human heavy chain gene segments, due to a variety of rearrangements, when employing either a mouse that expresses a light chain from only a human Vκ1-39- or a human Vκ3-20-derived light chain. Human V_H gene segments of the 2, 3, 4, and 5 families rearranged with a variety of human D_H segments and human J_H segments to yield antigen-specific antibodies.

[00143]

Table 3			
Vκ1-39Jκ5 Common Light Chain Antibodies			
Antibody	**HCVR**		
	V_H	**D_H**	**J_H**
2952	2-5	6-6	1
3022	3-23	3-10	4
3028	3-23	3-3	4
2955	3-30	6-6	1
3043	3-30	6-6	3
3014	3-30	1-7	4
3015	3-30	1-7	4
3023	3-30	1-7	4
3024	3-30	1-7	4
3032	3-30	1-7	4
3013	3-30	5-12	4
3042	3-30	5-12	4
2985	3-30	6-13	4
2997	3-30	6-13	4
3011	3-30	6-13	4
3047	3-30	6-13	4
3018	3-30	6-6	4
2948	3-30	7-27	4
2987	3-30	7-27	4
2996	3-30	7-27	4
3005	3-30	7-27	4
3012	3-30	7-27	4
3020	3-30	7-27	4
3021	3-30	7-27	4
3025	3-30	7-27	4
3030	3-30	7-27	4

3036	3-30	7-27	4
2982	3-30	3-22	5
2949	3-30	6-6	5
2950	3-30	6-6	5
2954	3-30	6-6	5
2978	3-30	6-6	5
3016	3-30	6-6	5
3017	3-30	6-6	5
3033	3-30	6-6	5
3041	3-30	6-6	5
3004	3-30	7-27	5
3010	4-59	3-16	3
3019	4-59	3-16	3
2964	4-59	3-22	3
3027	4-59	3-16	4
3046	5-51	5-5	3

[00144]

Table 4			
Vκ3-20Jκ1 Common Light Chain Antibodies			
Antibody	**HCVR**		
	V_H	D_H	J_H
2968	4-39	1-26	3
2975	5-51	6-13	5
2972	5-51	3-16	6

Example 7. Determination of Blocking Ability of Antigen-Specific Common Light Chain Antibodies by Luminex™ Assay

[00145] Ninety-eight human common light chain antibodies raised against Antigen E were tested for their ability to block binding of Antigen E's natural ligand (Ligand Y) to Antigen E in a bead-based assay.

[00146] The extracellular domain (ECD) of Antigen E was conjugated to two myc epitope tags and a 6X histidine tag (Antigen E-mmH) and amine-coupled to carboxylated microspheres at a concentration of 20 μg/mL in MES buffer. The mixture was incubated for two hours at room temperature followed by bead deactivation with 1M Tris pH 8.0 followed by washing in PBS with 0.05% (v/v) Tween-20. The beads were then blocked with PBS (Irvine Scientific, Santa Ana, CA) containing 2% (w/v) BSA (Sigma-Aldrich Corp., St. Louis,

MO). In a 96-well filter plate, supernatants containing Antigen E-specific common light chain antibodies, were diluted 1:15 in buffer. A negative control containing a mock supernatant with the same media components as for the antibody supernatant was prepared. Antigen E-labeled beads were added to the supernatants and incubated overnight at 4°C. Biotinylated-Ligand Y protein was added to a final concentration of 0.06 nM and incubated for two hours at room temperature. Detection of biotinylated-Ligand Y bound to Antigen E-myc-myc-6His labeled beads was determined with R-Phycoerythrin conjugated to Streptavidin (Moss Inc, Pasadena, MD) followed by measurement in a Luminex™ flow cytometry-based analyzer. Background Mean Fluorescence Intensity (MFI) of a sample without Ligand Y was subtracted from all samples. Percent blocking was calculated by division of the background-subtracted MFI of each sample by the adjusted negative control value, multiplying by 100 and subtracting the resulting value from 100.

[00147] In a similar experiment, the same 98 human common light chain antibodies raised against Antigen E were tested for their ability to block binding of Antigen E to Ligand Y-labeled beads.

[00148] Briefly, Ligand Y was amine-coupled to carboxylated microspheres at a concentration of 20 µg/mL diluted in MES buffer. The mixture and incubated two hours at room temperature followed by deactivation of beads with 1M Tris pH 8 then washing in PBS with 0.05% (v/v) Tween-20. The beads were then blocked with PBS (Irvine Scientific, Santa Ana, CA) containing 2% (w/v) BSA (Sigma-Aldrich Corp., St. Louis, MO). In a 96-well filter plate, supernatants containing Antigen E-specific common light chain antibodies were diluted 1:15 in buffer. A negative control containing a mock supernatant with the same media components as for the antibody supernatant was prepared. A biotinylated-Antigen E-mmH was added to a final concentration of 0.42 nM and incubated overnight at 4°C. Ligand Y-labeled beads were then added to the antibody/Antigen E mixture and incubated for two hours at room temperature. Detection of biotinylated-Antigen E-mmH bound to Ligand Y-beads was determined with R-Phycoerythrin conjugated to Streptavidin (Moss Inc, Pasadena, MD) followed by measurement in a Luminex™ flow cytometry-based analyzer. Background Mean Fluorescence Intensity (MFI) of a sample without Antigen E was subtracted from all samples. Percent blocking was calculated by division of the background-subtracted MFI of each sample by the adjusted negative control value, multiplying by 100 and subtracting the resulting value from 100.

[00149] Tables 5 and 6 show the percent blocking for all 98 anti-Antigen E common light chain antibodies tested in both Luminex™ assays. ND: not determined under current experimental conditions.

[00150] In the first Luminex™ experiment described above, 80 common light chain antibodies containing the Vκ1-39Jκ5 engineered light chain were tested for their ability to block Ligand Y binding to Antigen E-labeled beads. Of these 80 common light chain antibodies, 68 demonstrated >50% blocking, while 12 demonstrated <50% blocking (6 at 25-50% blocking and 6 at <25% blocking). For the 18 common light chain antibodies containing the Vκ3-20Jκ1 engineered light chain, 12 demonstrated >50% blocking, while 6 demonstrated <50% blocking (3 at 25-50% blocking and 3 at <25% blocking) of Ligand Y binding to Antigen E-labeled beads.

[00151] In the second Luminex™ experiment described above, the same 80 common light chain antibodies containing the Vκ1-39Jκ5 engineered light chain were tested for their ability to block binding of Antigen E to Ligand Y-labeled beads. Of these 80 common light chain antibodies, 36 demonstrated >50% blocking, while 44 demonstrated <50% blocking (27 at 25-50% blocking and 17 at <25% blocking). For the 18 common light chain antibodies containing the Vκ3-20Jκ1 engineered light chain, 1 demonstrated >50% blocking, while 17 demonstrated <50% blocking (5 at 25-50% blocking and 12 at <25% blocking) of Antigen E binding to Ligand Y-labeled beads.

[00152] The data of Tables 5 and 6 establish that the rearrangements described in Tables 3 and 4 generated anti-Antigen E-specific common light chain antibodies that blocked binding of Ligand Y to its cognate receptor Antigen E with varying degrees of efficacy, which is consistent with the anti-Antigen E common light chain antibodies of Tables 3 and 4 comprising antibodies with overlapping and non-overlapping epitope specificity with respect to Antigen E.

[00153]

Table 5		
Vκ1-39Jκ5 Common Light Chain Antibodies		
Antibody	**% Blocking of Antigen E-Labeled Beads**	**% Blocking of Antigen E In Solution**
2948	81.1	47.8
2948G	38.6	ND
2949	97.6	78.8
2949G	97.1	73.7
2950	96.2	81.9
2950G	89.8	31.4
2952	96.1	74.3
2952G	93.5	39.9

2954	93.7	70.1
2954G	91.7	30.1
2955	75.8	30.0
2955G	71.8	ND
2964	92.1	31.4
2964G	94.6	43.0
2978	98.0	95.1
2978G	13.9	94.1
2982	92.8	78.5
2982G	41.9	52.4
2985	39.5	31.2
2985G	2.0	5.0
2987	81.7	67.8
2987G	26.6	29.3
2996	87.3	55.3
2996G	95.9	38.4
2997	93.4	70.6
2997G	9.7	7.5
3004	79.0	48.4
3004G	60.3	40.7
3005	97.4	93.5
3005G	77.5	75.6
3010	98.0	82.6
3010G	97.9	81.0
3011	87.4	42.8
3011G	83.5	41.7
3012	91.0	60.8
3012G	52.4	16.8
3013	80.3	65.8
3013G	17.5	15.4
3014	63.4	20.7
3014G	74.4	28.5
3015	89.1	55.7
3015G	58.8	17.3
3016	97.1	81.6
3016G	93.1	66.4
3017	94.8	70.2
3017G	87.9	40.8
3018	85.4	54.0
3018G	26.1	12.7

3019	99.3	92.4
3019G	99.3	88.1
3020	96.7	90.3
3020G	85.2	41.5
3021	74.5	26.1
3021G	81.1	27.4
3022	65.2	17.6
3022G	67.2	9.1
3023	71.4	28.5
3023G	73.8	29.7
3024	73.9	32.6
3024G	89.0	10.0
3025	70.7	15.6
3025G	76.7	24.3
3027	96.2	61.6
3027G	98.6	75.3
3028	92.4	29.0
3028G	87.3	28.8
3030	6.0	10.6
3030G	41.3	14.2
3032	76.5	31.4
3032G	17.7	11.0
3033	98.2	86.1
3033G	93.6	64.0
3036	74.7	32.7
3036G	90.1	51.2
3041	95.3	75.9
3041G	92.4	51.6
3042	88.1	73.3
3042G	60.9	25.2
3043	90.8	65.8
3043G	92.8	60.3

[00154]

Table 6		
Vκ3-20Jκ1 Common Light Chain Antibodies		
Antibody	% Blocking of Antigen E-Labeled Beads	% Blocking of Antigen E In Solution
2968	97.1	73.3
2968G	67.1	14.6
2969	51.7	20.3
2969G	37.2	16.5
2970	92.2	34.2
2970G	92.7	27.2
2971	23.4	11.6
2971G	18.8	18.9
2972	67.1	38.8
2972G	64.5	39.2
2973	77.7	27.0
2973G	51.1	20.7
2974	57.8	12.4
2974G	69.9	17.6
2975	49.4	18.2
2975G	32.0	19.5
2976	1.0	1.0
2976G	50.4	20.4

Example 8. Determination of Blocking Ability of Antigen-Specific Common Light Chain Antibodies by ELISA

[00155] Human common light chain antibodies raised against Antigen E were tested for their ability to block Antigen E binding to a Ligand Y-coated surface in an ELISA assay.

[00156] Ligand Y was coated onto 96-well plates at a concentration of 2 μg/mL diluted in PBS and incubated overnight followed by washing four times in PBS with 0.05% Tween-20. The plate was then blocked with PBS (Irvine Scientific, Santa Ana, CA) containing 0.5% (w/v) BSA (Sigma-Aldrich Corp., St. Louis, MO) for one hour at room temperature. In a separate plate, supernatants containing anti-Antigen E common light chain antibodies were diluted 1:10 in buffer. A mock supernatant with the same components of the antibodies was used as a negative control. Antigen E-mmH (described above) was added to a final concentration of 0.150 nM and incubated for one hour at room temperature. The

antibody/Antigen E-mmH mixture was then added to the plate containing Ligand Y and incubated for one hour at room temperature. Detection of Antigen E-mmH bound to Ligand Y was determined with Horse-Radish Peroxidase (HRP) conjugated to anti-Penta-His antibody (Qiagen, Valencia, CA) and developed by standard colorimetric response using tetramethylbenzidine (TMB) substrate (BD Biosciences, San Jose, CA) neutralized by sulfuric acid. Absorbance was read at OD450 for 0.1 sec. Background absorbance of a sample without Antigen E was subtracted from all samples. Percent blocking was calculated by division of the background-subtracted MFI of each sample by the adjusted negative control value, multiplying by 100 and subtracting the resulting value from 100.

[00157] Tables 7 and 8 show the percent blocking for all 98 anti-Antigen E common light chain antibodies tested in the ELISA assay. ND: not determined under current experimental conditions.

[00158] As described in this Example, of the 80 common light chain antibodies containing the Vκ1-39Jκ5 engineered light chain tested for their ability to block Antigen E binding to a Ligand Y-coated surface, 22 demonstrated >50% blocking, while 58 demonstrated <50% blocking (20 at 25-50% blocking and 38 at <25% blocking). For the 18 common light chain antibodies containing the Vκ3-20Jκ1 engineered light chain, 1 demonstrated >50% blocking, while 17 demonstrated <50% blocking (5 at 25-50% blocking and 12 at <25% blocking) of Antigen E binding to a Ligand Y-coated surface.

[00159] These results are also consistent with the Antigen E-specific common light chain antibody pool comprising antibodies with overlapping and non-overlapping epitope specificity with respect to Antigen E.

[00160]

Table 7	
Vκ1-39Jκ5 Common Light Chain Antibodies	
Antibody	**% Blocking of Antigen E In Solution**
2948	21.8
2948G	22.9
2949	79.5
2949G	71.5
2950	80.4
2950G	30.9
2952	66.9
2952G	47.3

2954	55.9
2954G	44.7
2955	12.1
2955G	25.6
2964	34.8
2964G	47.7
2978	90.0
2978G	90.2
2982	59.0
2982G	20.4
2985	10.5
2985G	ND
2987	31.4
2987G	ND
2996	29.3
2996G	ND
2997	48.7
2997G	ND
3004	16.7
3004G	3.5
3005	87.2
3005G	54.3
3010	74.5
3010G	84.6
3011	19.4
3011G	ND
3012	45.0
3012G	12.6
3013	39.0
3013G	9.6
3014	5.2
3014G	17.1
3015	23.7
3015G	10.2
3016	78.1
3016G	37.4
3017	61.6
3017G	25.2
3018	40.6
3018G	14.5

3019	94.6
3019G	92.3
3020	80.8
3020G	ND
3021	7.6
3021G	20.7
3022	2.4
3022G	15.0
3023	9.1
3023G	19.2
3024	7.5
3024G	15.2
3025	ND
3025G	13.9
3027	61.4
3027G	82.7
3028	40.3
3028G	12.3
3030	ND
3030G	9.5
3032	ND
3032G	13.1
3033	77.1
3033G	32.9
3036	17.6
3036G	24.6
3041	59.3
3041G	30.7
3042	39.9
3042G	16.1
3043	57.4
3043G	46.1

[00161]

Table 8	
Vκ3-20Jκ1 Common Light Chain Antibodies	
Antibody	% Blocking of Antigen E In Solution
2968	68.9
2968G	15.2
2969	10.1
2969G	23.6
2970	34.3
2970G	41.3
2971	6.3
2971G	27.1
2972	9.6
2972G	35.7
2973	20.7
2973G	23.1
2974	ND
2974G	22.0
2975	8.7
2975G	19.2
2976	4.6
2976G	26.7

Example 9. BIAcore™ Affinity Determination for Antigen-Specific Common Light Chain Antibodies

[00162] Equilibrium dissociation constants (K_D) for selected antibody supernatants were determined by SPR (Surface Plasmon Resonance) using a BIAcore™ T100 instrument (GE Healthcare). All data was obtained using HBS-EP (10mM Hepes, 150mM NaCl, 0.3mM EDTA, 0.05% Surfactant P20, pH 7.4) as both the running and sample buffers, at 25°C. Antibodies were captured from crude supernatant samples on a CM5 sensor chip surface previously derivatized with a high density of anti-human Fc antibodies using standard amine coupling chemistry. During the capture step, supernatants were injected across the anti-human Fc surface at a flow rate of 3 μL/min, for a total of 3 minutes. The capture step was followed by an injection of either running buffer or analyte at a concentration of 100 nM for 2 minutes at a flow rate of 35 μL/min. Dissociation of antigen from the captured antibody was monitored for 6 minutes. The captured antibody was removed by a brief injection of 10 mM

glycine, pH 1.5. All sensorgrams were double referenced by subtracting sensorgrams from buffer injections from the analyte sensorgrams, thereby removing artifacts caused by dissociation of the antibody from the capture surface. Binding data for each antibody was fit to a 1:1 binding model with mass transport using BIAcore T100 Evaluation software v2.1. Results are shown in Tables 9 and 10.

[00163] The binding affinities of common light chain antibodies comprising the rearrangements shown in Tables 3 and 4 vary, with nearly all exhibiting a K_D in the nanomolar range. The affinity data is consistent with the common light chain antibodies resulting from the combinatorial association of rearranged variable domains described in Tables 3 and 4 being high-affinity, clonally selected, and somatically mutated. Coupled with data previously shown, the common light chain antibodies described in Tables 3 and 4 comprise a collection of diverse, high-affinity antibodies that exhibit specificity for one or more epitopes on Antigen E.

[00164]

Table 9		
Vκ1-39Jκ5 Common Light Chain Antibodies		
Antibody	**100nM Antigen E**	
	K_D (nM)	**$T_{1/2}$ (min)**
2948	8.83	28
2948G	95.0	1
2949	3.57	18
2949G	6.37	9
2950	4.91	17
2950G	13.6	5
2952	6.25	7
2952G	7.16	4
2954	2.37	24
2954G	5.30	9
2955	14.4	6
2955G	12.0	4
2964	14.8	6
2964G	13.0	9
2978	1.91	49
2978G	1.80	58
2982	6.41	19

2982G	16.3	9
2985	64.4	9
2985G	2.44	8
2987	21.0	11
2987G	37.6	4
2996	10.8	9
2996G	24.0	2
2997	7.75	19
2997G	151	1
3004	46.5	14
3004G	1.93	91
3005	2.35	108
3005G	6.96	27
3010	4.13	26
3010G	2.10	49
3011	59.1	5
3011G	41.7	5
3012	9.71	20
3012G	89.9	2
3013	20.2	20
3013G	13.2	4
3014	213	4
3014G	36.8	3
3015	29.1	11
3015G	65.9	0
3016	4.99	17
3016G	18.9	4
3017	9.83	8
3017G	55.4	2
3018	11.3	36
3018G	32.5	3
3019	1.54	59
3019G	2.29	42
3020	5.41	39
3020G	41.9	6
3021	50.1	6
3021G	26.8	4
3022	25.7	17
3022G	20.8	12
3023	263	9

3023G	103	5
3024	58.8	7
3024G	7.09	10
3025	35.2	6
3025G	42.5	8
3027	7.15	6
3027G	4.24	18
3028	6.89	37
3028G	7.23	22
3030	46.2	7
3030G	128	3
3032	53.2	9
3032G	13.0	1
3033	4.61	17
3033G	12.0	5
3036	284	12
3036G	18.2	10
3041	6.90	12
3041G	22.9	2
3042	9.46	34
3042G	85.5	3
3043	9.26	29
3043G	13.1	22

[00165]

Table 10		
Vκ3-20Jκ1 Common Light Chain Antibodies		
Antibody	100nM Antigen E	
	K_D (nM)	$T_{1/2}$ (min)
2968	5.50	8
2968G	305	0
2969	34.9	2
2969G	181	1
2970G	12.3	3
2971G	32.8	22
2972	6.02	13
2972G	74.6	26
2973	5.35	39
2973G	11.0	44

2974	256	0
2974G	138	0
2975	38.0	2
2975G	134	1
2976	6.73	10
2976G	656	8

Example 10. Determination of Binding Specificities of Antigen-Specific Common Light Chain Antibodies by Luminex™ Assay

[00166] Selected anti-Antigen E common light chain antibodies were tested for their ability to bind to the ECD of Antigen E and Antigen E ECD variants, including the cynomolgous monkey ortholog (*Mf* Antigen E), which differs from the human protein in approximately 10% of its amino acid residues; a deletion mutant of Antigen E lacking the last 10 amino acids from the C-terminal end of the ECD (Antigen E-ΔCT); and two mutants containing an alanine substitution at suspected locations of interaction with Ligand Y (Antigen E-Ala1 and AntigenE-Ala2). The Antigen E proteins were produced in CHO cells and each contained a myc-myc-His C-terminal tag.

[00167] For the binding studies, Antigen E ECD protein or variant protein (described above) from 1 mL of culture medium was captured by incubation for 2 hr at room temperature with 1 x 10^6 microsphere (Luminex™) beads covalently coated with an anti-myc monoclonal antibody (MAb 9E10, hybridoma cell line CRL-1729™; ATCC, Manassas, VA). The beads were then washed with PBS before use. Supernatants containing anti-Antigen E common light chain antibodies were diluted 1:4 in buffer and added to 96-well filter plates. A mock supernatant with no antibody was used as negative control. The beads containing the captured Antigen E proteins were then added to the antibody samples (3000 beads per well) and incubated overnight at 4°C. The following day, the sample beads were washed and the bound common light chain antibody was detected with a R-phycoerythrin-conjugated anti-human IgG antibody. The fluorescence intensity of the beads (approximately 100 beads counted for each antibody sample binding to each Antigen E protein) was measured with a Luminex™ flow cytometry-based analyzer, and the median fluorescence intensity (MFI) for at least 100 counted beads per bead/antibody interaction was recorded. Results are shown in Tables 11 and 12.

[00168]

Table 11					
Vκ1-39Jκ5 Common Light Chain Antibodies					
Antibody	Mean Fluorescence Intensity (MFI)				
	Antigen E-ECD	Antigen E-ΔCT	Antigen E-Ala1	Antigen E-Ala2	*Mf* Antigen E
2948	1503	2746	4953	3579	1648
2948G	537	662	2581	2150	863
2949	3706	4345	8169	5678	5142
2949G	3403	3318	7918	5826	5514
2950	3296	4292	7756	5171	4749
2950G	2521	2408	7532	5079	3455
2952	3384	1619	1269	168	911
2952G	3358	1001	108	55	244
2954	2808	3815	7114	5039	3396
2954G	2643	2711	7620	5406	3499
2955	1310	2472	4738	3765	1637
2955G	1324	1802	4910	3755	1623
2964	5108	1125	4185	346	44
2964G	4999	729	4646	534	91
2978	6986	2800	14542	10674	8049
2978G	5464	3295	11652	8026	6452
2982	4955	2388	13200	9490	6772
2982G	3222	2013	8672	6509	4949
2985	1358	832	4986	3892	1669
2985G	43	43	128	244	116
2987	3117	1674	7646	5944	2546
2987G	3068	1537	9202	6004	4744
2996	4666	1917	12875	9046	6459
2996G	2752	1736	8742	6150	4873
2997	5164	2159	12167	8361	5922
2997G	658	356	3392	2325	1020
3004	2794	1397	8542	6268	3083
3004G	2753	1508	8267	5808	4345
3005	5683	2221	12900	9864	5868
3005G	4344	2732	10669	7125	5880
3010	4829	1617	2642	3887	44
3010G	3685	1097	2540	3022	51

3011	2859	2015	7855	5513	3863
3011G	2005	1072	6194	4041	3181
3012	3233	2221	8543	5637	3307
3012G	968	378	3115	2261	1198
3013	2343	1791	6715	4810	2528
3013G	327	144	1333	1225	370
3014	1225	1089	5436	3621	1718
3014G	1585	851	5178	3705	2411
3015	3202	2068	8262	5554	3796
3015G	1243	531	4246	2643	1611
3016	4220	2543	8920	5999	5666
3016G	2519	1277	6344	4288	4091
3017	3545	2553	8700	5547	5098
3017G	1972	1081	5763	3825	3038
3018	2339	1971	6140	4515	2293
3018G	254	118	978	1020	345
3019	5235	1882	7108	4249	54
3019G	4090	1270	4769	3474	214
3020	3883	3107	8591	6602	4420
3020G	2165	1209	6489	4295	2912
3021	1961	1472	6872	4641	2742
3021G	2091	1005	6430	3988	2935
3022	2418	793	7523	2679	36
3022G	2189	831	6182	3051	132
3023	1692	1411	5788	3898	2054
3023G	1770	825	5702	3677	2648
3024	1819	1467	6179	4557	2450
3024G	100	87	268	433	131
3025	1853	1233	6413	4337	2581
3025G	1782	791	5773	3871	2717
3027	4131	1018	582	2510	22
3027G	3492	814	1933	2596	42
3028	4361	2545	9884	5639	975
3028G	2835	1398	7124	3885	597
3030	463	277	1266	1130	391
3030G	943	302	3420	2570	1186
3032	2083	1496	6594	4402	2405
3032G	295	106	814	902	292
3033	4409	2774	8971	6331	5825
3033G	2499	1234	6745	4174	4210

3036	1755	1362	6137	4041	1987
3036G	2313	1073	6387	4243	3173
3041	3674	2655	8629	5837	4082
3041G	2519	1265	6468	4274	3320
3042	2653	2137	7277	5124	3325
3042G	1117	463	4205	2762	1519
3043	3036	2128	7607	5532	3366
3043G	2293	1319	6573	4403	3228

[00169]

Table 12					
Vκ3-20Jκ1 Common Light Chain Antibodies					
Antibody	**Mean Fluorescence Intensity (MFI)**				
	Antigen E-ECD	**Antigen E-ΔCT**	**Antigen E-Ala1**	**Antigen E-Ala2**	***Mf* Antigen E**
2968	6559	3454	14662	3388	29
2968G	2149	375	9109	129	22
2969	2014	1857	7509	5671	3021
2969G	1347	610	6133	4942	2513
2970	5518	1324	14214	607	32
2970G	4683	599	12321	506	31
2971	501	490	2506	2017	754
2971G	578	265	2457	2062	724
2972	2164	2158	8408	6409	3166
2972G	1730	992	6364	4602	2146
2973	3527	1148	3967	44	84
2973G	1294	276	1603	28	44
2974	1766	722	8821	241	19
2974G	2036	228	8172	135	26
2975	1990	1476	8669	6134	2468
2975G	890	315	4194	3987	1376
2976	147	140	996	1079	181
2976G	1365	460	6024	3929	1625

[00170] The anti-Antigen E common light chain antibody supernatants exhibited high specific binding to the beads linked to Antigen E-ECD. For these beads, the negative control mock supernatant resulted in negligible signal (<10 MFI) when combined with the Antigen E-ECD bead sample, whereas the supernatants containing anti-Antigen E common

light chain antibodies exhibited strong binding signal (average MFI of 2627 for 98 antibody supernatants; MFI > 500 for 91/98 antibody samples).

[00171] As a measure of the ability of the selected anti-Antigen E common light chain antibodies to identify different epitopes on the ECD of Antigen E, the relative binding of the antibodies to the variants were determined. All four Antigen E variants were captured to the anti-myc Luminex™ beads as described above for the native Antigen E-ECD binding studies, and the relative binding ratios ($MFI_{variant}/MFI_{Antigen\ E\text{-}ECD}$) were determined. For 98 tested common light chain antibody supernatants shown in Tables 11 and 12, the average ratios ($MFI_{variant}/MFI_{Antigen\ E\text{-}ECD}$) differed for each variant, likely reflecting different capture amounts of proteins on the beads (average ratios of 0.61, 2.9, 2.0, and 1.0 for Antigen E-ΔCT, Antigen E-Ala1, Antigen E-Ala2, and *Mf* Antigen E, respectively). For each protein variant, the binding for a subset of the 98 tested common light chain antibodies showed greatly reduced binding, indicating sensitivity to the mutation that characterized a given variant. For example, 19 of the common light chain antibody samples bound to the *Mf* Antigen E with $MFI_{variant}/MFI_{Antigen\ E\text{-}ECD}$ of <8%. Since many in this group include high or moderately high affinity antibodies (5 with K_D < 5nM, 15 with K_D < 50 nM), it is likely that the lower signal for this group results from sensitivity to the sequence (epitope) differences between native Antigen E-ECD and a given variant rather than from lower affinities.

[00172] These data establish that the common light chain antibodies described in Tables 3 and 4 indeed represent a diverse group of Antigen-E-specific common light chain antibodies that specifically recognize more than one epitope on Antigen E.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A mouse, comprising:

(a) a single rearranged human immunoglobulin light chain variable region (V_L/J_L) that encodes a human V_L domain of an immunoglobulin light chain, wherein the single rearranged human V_L/J_L region is selected from a human Vκ1-39/J gene segment or a human Vκ3-20/J gene segment; and,

(b) a replacement of endogenous heavy chain variable (V_H) gene segments with one or more human V_H gene segments, wherein the human V_H gene segments are operably linked to an endogenous heavy chain constant (C_H) region gene, and the human V_H gene segments are capable of rearranging and forming a human/mouse chimeric heavy chain gene.

2. The mouse of claim 1, wherein the mouse comprises an immunoglobulin light chain variable region locus that does not comprise an endogenous V_L gene segment that is capable of rearranging and forming an immunoglobulin light chain gene.

3. The mouse of claim 1 or claim 2, further comprising a mouse κ intronic enhancer and/or a mouse κ 3' enhancer.

4. The mouse of any one of claims 1 to 3, wherein the single rearranged human immunoglobulin light chain variable region (V_L/J_L) is operably linked to a mouse C_L region.

5. The mouse of claim 4, wherein the C_L region is a Cκ region.

6. The mouse of any one of claims 1 to 5, wherein the one or more human V_H gene segments comprise at least 19, at least 39, at least 80, or 81 unrearranged human V_H gene segments.

7. The mouse of claims 1 to 5, wherein the one or more human V_H gene segments comprise at least 12, at least 25, or at least 43 functional human V_H gene segments.

8. The mouse of claim 1, 6 or 7, wherein the one or more human V_H gene segments are selected from 1-2, 1-8, 1-24, 2-5, 3-7, 3-9, 3-11, 3-13, 3-15, 3-20, 3-23, 3-30, 3-33, 3-48, 4-31, 4-39, 4-59, 5-51, 6-1 and a combination thereof.

9. The mouse of any one of claims 1 to 8, further comprising at least one unrearranged human D gene segment and at least one unrearranged human J gene segment.

10. The mouse of claim 9, wherein the at least one unrearranged human D gene segments are selected from D1-7, D1-26, D3-3, D3-10, D3-16, D3-22, D5-5, D5-12, D6-6, D6-13, D7-27, and a combination thereof.

11. The mouse of claim 9, wherein the at least one unrearranged human J gene segment is selected from J1, J3, J4, J5, J6, and a combination thereof.

12. The mouse of claim 1, wherein the mouse comprises a B cell that comprises a rearranged immunoglobulin heavy chain variable region gene sequence comprising a human heavy chain variable region gene derived from a V_H gene segment selected from a V_H2-5, V_H3-23, V_H3-30, V_H4-39, V_H4-59, and V_H5-51, and derived from a D gene segment selected from a D1-7, D1-26, D3-3, D3-16, D3-10, D3-22, D5-5, D5-12, D6-6, D6-13, and D7-27.

13. The mouse of claim 1, wherein the human Vκ1-39 gene segment is present in a rearrangement with a human Jκ5 gene segment.

14. The mouse of claim 1, wherein the human Vκ3-20 gene segment is present in a rearrangement with a human Jκ1 gene segment.

15. An isolated cell from the mouse of claim 1.

16. The cell of claim 15, wherein the cell is an embryonic stem (ES) cell.

17. The cell of claim 15, wherein the cell is a lymphocyte.

18. The cell of claim 17, wherein the lymphocyte is a B cell.

19. A hybridoma made with the B cell of claim 18.

20. A reverse chimeric antibody made by the mouse of any one of claims 1 to 14, wherein the reverse chimeric antibody comprises a light chain comprising a human V_L and a mouse C_L, and a heavy chain comprising a human V_H and a mouse C_H.