SYSTEMS AND METHODS TO PRODUCE B CELLS GENETICALLY MODIFIED TO EXPRESS SELECTED ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application No. 62/575,275 filed October 20, 2017, to US Provisional Patent Application No. 62/580,303 filed November 1, 2017, and to US Provisional Patent Application No. 62/623,371 filed January 29, 2018, each of which is incorporated herein by reference in its entirety as if fully set forth herein.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 18-024-WO-PCT_ST25.txt. The text file is 184 KB, was created on October 19, 2018, and is being submitted electronically via EFS-Web.

FIELD OF THE DISCLOSURE

[0003] The current disclosure provides systems and methods to genetically modify B cells to express selected antibodies. The systems and methods can be used to: obviate the need for classical vaccinations; provide protection against infectious agents for which no vaccinations are currently available; provide protection against infectious agents when patients are otherwise immune-suppressed; and/or provide a benefit provided by a therapeutic antibody, such as in the treatment of autoimmune disorders.

BACKGROUND OF THE DISCLOSURE

[0004] Vaccines are designed to increase the immunity of a subject against a particular infection by stimulating B cells to produce antibodies against the targeted infectious agent. Routine pediatric vaccination is a long established clinical intervention with comparatively low risk and high efficacy. Unfortunately, however, vaccinations are not available for all infectious agents. As one example, every year in the United States, millions of children visit a doctor or emergency room due to infections with Respiratory Syncytial Virus (RSV).

[0005] For decades, researchers have been trying to develop a vaccine that can induce B cells to produce antibodies that are effective to protect against viruses such as RSV, human immunodeficiency virus (HIV), and Zika virus. But all efforts to induce protective antibodies have failed. The only RSV vaccine tested widely actually made infection worse: antibodies generated following vaccination did not disable the virus, but instead, enhanced its ability to infect cells. Besides RSV, HIV, and Zika virus there are a number of other infectious agents for which no

effective vaccines are available.

[0006] In addition to combating infections, antibodies can also be useful as treatments for other conditions such as autoimmune diseases. However, these antibody-based therapies typically require repeated injections of the antibodies to maintain protection.

[0007] Also of note, numerous patients undergo bone marrow or hematopoietic stem cell transplants as treatments for hematological malignancies (e.g., leukemia, lymphoma, myeloma). Other patients receive infusions of genetically-modified hematopoietic stem cells that provide a therapeutic gene that the patient lacks. All of these treatments require that the patient's existing immune system be removed before administration of the transplant or genetically-modified hematopoietic stem cells, leaving a dangerous window of immune suppression before the patient's immune system repopulates following the treatment. During this time of immune suppression, patients are incredibly susceptible to infections, such as RSV, influenza, parainfluenza, and metapneumovirus (MPV). These infections are a high risk factor and associated with numerous fatalities following these treatments.

SUMMARY OF THE DISCLOSURE

[0008] The current disclosure provides systems and methods to genetically modify B cells to express selected antibodies. In particular embodiments, the selected antibodies reduce or obviate the need for existing vaccinations. In particular embodiments, the selected antibodies protect against infection from viruses for which no effective vaccination strategies are currently available (e.g., RSV, HIV, Zika). In particular embodiments, the selected antibodies reduce or obviate the need for therapeutic antibody injections, such as those administered to treat various autoimmune disorders. In particular embodiments, the selected antibodies protect immune-suppressed patients from infections. In particular embodiments, methods of the disclosure can be used to reprogram B cells to protect against hundreds of different infectious agents or pathogens, all via a single laboratory manipulation encompassing a few days.

[0009] In particular embodiments, the current disclosure provides these benefits through the targeted insertion of a genetic construct into a particular area of the B cell's endogenous genome. Importantly, the genetic modification of B cells is difficult due to the high variability of genetic sequences within these cells that are required for antibody diversity. This high degree of genetic variability makes directly targeting antibody coding regions for genetic manipulation impractical. Moreover, removing and replacing coding portions of the B cell's genome is also not effective because this approach negatively affects B cell function.

[0010] Additional challenges regarding genetically modifying B cells to express selected

antibodies arise because antibodies are formed from discrete protein units, referred to as heavy chains and light chains. The different chains are encoded by different portions of the B cell genome, yet must come together to form a functioning antibody.

[0011] The current disclosure overcomes the noted challenges, among others, by identifying a constant region of the B cell genome that can be reliably targeted for genetic insertion and that, when modified, results in preferential expression of an inserted genetic construct over corresponding portions of the B cell's natural genome. This strategy overcomes sequence variability associated with the B cell genome and also overcomes the need to remove and replace portions of the endogenous B cell genome to achieve functional expression of the selected antibody. Overcoming the need to remove and replace portions of the endogenous B cell genome preserves B cell function after the genetic manipulation.

[0012] In particular embodiments, the noted area targeted for genetic insertion is an intronic region upstream or downstream of an Eμ enhancer element of SEQ ID NO: 85 (human) or SEQ ID NO: 86 (mouse). In particular embodiments, the area targeted for genetic insertion is a constant intronic region selected from SEQ ID NO: 1 or 2 (human) or SEQ ID NO: 3 or 4 (mouse). In particular embodiments, human DNA sequences within SEQ ID NO: 1 to target for genetic insertion include SEQ ID NOs: 5-24. In particular embodiments, human DNA sequences within SEQ ID NO: 2 to target for genetic insertion include SEQ ID NOs: 25-44. In particular embodiments, mouse DNA sequences within SEQ ID NO: 3 to target for genetic insertion include SEQ ID NOs: 45-64. In particular embodiments, mouse DNA sequences within SEQ ID NO: 4 to target for genetic insertion include SEQ ID NOs: 65-84. Genetic sequences particularly capable of targeting these sites for genetic modification are described within the current disclosure as guide RNA (gRNA) SEQ ID NOs: 87-89, and 290-366.

[0013] In particular embodiments, the placement and components of an inserted genetic construct result in preferential expression of the inserted genetic construct over corresponding portions of the B cell's endogenous genome. These embodiments also include elements that overcome challenges associated with portions of antibodies being encoded by different regions of the endogenous B cell genome.

[0014] In particular embodiments, the genetic constructs are inserted into one of SEQ ID NO: 1, 2, 3, and 4 and include (i) a promoter; (ii) a signal peptide; (iii) a transgene encoding an entire light chain of a selected antibody; (iv) a flexible linker or a skipping element; (v) the variable portion of the heavy chain of a selected antibody; and (vi) a splice junction that results in expression of the B cell's endogenous heavy chain constant region. In these embodiments, expressing the selected antibody as a single construct overcomes challenges associated with portions of

antibodies being encoded by different areas of the endogenous B cell genome. Inclusion of a flexible linker physically links the light chain portion and the heavy chain portion of the expressed selected antibody in a manner that allows them to form a functional unit and at the same time reduces the risk of the antibody portions binding with other potentially expressed antibody chains from the B cell's endogenous genome. Use of a skipping element does not physically link the light chain portion and the heavy chain portion, but their expression in close proximity also results in association to form a functional unit while at the same time reducing the risk of the antibody portions binding with other potentially expressed antibody chains from the B cell's endogenous genome. Inclusion of a splice junction results in the selected antibody including a heavy chain constant region appropriate for the B cell's current activation and/or maturation state. In other words, the selected expressed antibodies can be expressed having any of the B cell's endogenous heavy chain constant regions, and the heavy chain constant region expressed with the selected antibody can naturally change over time.

[0015] The current disclosure also provides methods to ensure that only B cells that have been effectively genetically modified to express a selected antibody are collected for formulation and administration to patients. For example, before genetic modification, a B cell will naturally express antibodies that include either a kappa light chain or a lambda light chain. The B cell can be modified to express a light chain that is different from the kappa or lambda chain that it naturally expresses, and only those B cells that express the replacement chain are selected for formulation and administration.

[0016] The current disclosure also provides numerous additional strategies to effectively modify B cells to provide the benefits described herein. These and other strategies are described more fully in the Detailed Description below.

BRIEF DESCRIPTION OF THE FIGURES

[0017] FIG. 1. Schematic of B cell response to classical vaccination strategies.

[0018] FIGs. 2A-2C. Schematics of prior art vaccination strategies against Respiratory Syncytial Virus (RSV): (FIG. 2A) formalin-inactivated RSV vaccine; (FIG. 2B) "improved" RSV vaccines; and (FIG. 2C) RSV epitope scaffold vaccine.

[0019] FIGs. 3A-3C. Summary of previous efforts to bypass vaccination and directly provide protective antibodies against RSV: (FIG. 3A) palivizumab injection; (FIG. 3B) adenovirus-mediated palivizumab expression; and (FIG. 3C) stem cell genetic modification and differentiation.

[0020] FIGs. 4A, 4B. Schematics for particular embodiments of protection strategies disclosed herein: (FIG. 4A) B cell modification to protect against RSV; and (FIG. 4B) strategy for

simultaneous protection.

[0021] FIG. 5. Hypothesized secretion of palivizumab by B cell subtypes in the presence and absence of infection.

[0022] FIGs. 6A, 6B. The structure of (FIG. 6A) antibody genes and (FIG. 6B) antibody proteins, highlighting a technical challenge of the disclosure: that antibodies are proteins made from two separate gene products. In particular embodiments, synthetic genetic constructs encoding selected antibodies disclosed herein utilize skipping elements (e.g., self-cleaving peptides) to address this challenge.

[0023] FIG. 7. Schematic depicting an endogenous heavy chain gene locus, including V, D, and J segments that recombine, enhancer elements, shown as a circle and an oval, and 6 potential constant regions that can be expressed. B cells start by expressing the μ/δ constant regions, but can switch to using γ, α, or ε constant regions by deleting intervening DNA. Also note that each V segment is associated with a heavy chain promoter denoted by an arrow that drives expression of the heavy chain following recombination.

[0024] FIGs. 8A, 8B. Because two proteins come together to form an antibody, it can be desirable to target or inactivate the B cell's endogenous antibody heavy chain (IgH) and/or endogenous antibody light chain (IgL). In the absence of such targeting or inactivation, undesired hybrid antibodies could form (i.e., an endogenous light chain pairing with a selected antibody heavy chain or vice versa). (FIG. 8A) An approach for endogenous IgH targeting and a resulting chimera including portions of a selected antibody (e.g., Palivizumab). (FIG. 8B) An approach for IgL inactivation. In the depicted approaches, a stop codon can be placed upstream (or as part of) the inserted genetic construct.

[0025] FIG. 9. Schematic depicting interactions of heavy chain enhancers with (top) an endogenous VDJ or (bottom) a synthetic VDJ encoded by an inserted genetic construct. Promoters are depicted as arrows. Nucleic acid is depicted as boxes. B cells naturally express nucleic acid that is downstream of the promoter that is closest to the Eμ enhancer. In the endogenous B cell genome depicted in FIG. 9, the first upstream promoter closest to the Eμ enhancer then drives expression of the endogenous heavy chain VDJ segments. Inserting a genetic construct that includes a promoter between the Eμ enhancer and the first endogenous promoter, results in the B cell expressing the inserted genetic construct rather than the endogenous heavy chain VDJ segments. This inserted gene could be a VDJ variable region of a heavy chain, a paired full antibody light chain together with the heavy chain variable VDJ, or another synthetic gene capable of being expressed as a fusion with a B cell heavy chain constant region. In FIG. 9, the constant regions are individually denoted to highlight that the inserted

genetic construct can be expressed with any of the potential heavy chain constant regions.

[0026] FIG. 10. Target regions for insertion (Human). Regions for genetic construct insertion into the genome: #1: From the terminal J region (IGHJ6 in human, IGHJ4 in mouse) to the Eμ enhancer, or #2: From the Eμ enhancer to the repetitive sequences of the constant domain switch region.

[0027] FIG. 11A, 11B. (FIG. 11A) the human Eμ intronic enhancer (SEQ ID NO: 85) and human DNA sequence to target for genetic insertion including from IGHJ6 to Eμ intronic enhancer (SEQ ID NO: 1); (FIG. 11B) exemplary associated gRNA (e.g., sgRNA) target sites (SEQ ID NOs. 5-24) and gRNA sequences (SEQ ID NOs: 88, 89, and 290-307).

[0028] FIGs. 12A, 12B. (FIG. 12A) Human DNA sequence to target for genetic insertion including from Eμ intronic enhancer to switch region (SEQ ID NO: 2); (FIG. 12B) exemplary associated gRNA (e.g., sgRNA) target sites (SEQ ID NOs. 25-44) and gRNA sequences (SEQ ID NOs: 308-327).

[0029] FIGs. 13A, 13B. (FIG. 13A) the mouse Eμ intronic enhancer (SEQ ID NO: 86) and mouse DNA sequence to target for genetic insertion including from IGHJ4 to Eμ intronic enhancer (SEQ ID NO: 3); (FIG. 13B) exemplary associated gRNA (e.g., sgRNA) target sites (SEQ ID NOs. 45-64) and gRNA sequences (SEQ ID NOs: 87, and 328-346).

[0030] FIGs. 14A, 14B. (FIG. 14A) Mouse DNA sequence to target for genetic insertion including from Eμ intronic enhancer to switch region (SEQ ID NO: 4); (FIG. 14B) exemplary associated gRNA (e.g., sgRNA) target sites (SEQ ID NOs. 65-84) and gRNA sequences (SEQ ID NOs: 347-366).

[0031] FIGs. 15A, 15B. FIG. 15A is a schematic depicting insertion of a genetic construct encoding an anti-RSV antibody into an endogenous heavy chain locus, utilizing a CRISPR/Cas9 gene-editing system. The genetic construct can include homology arms or stitches, which are nucleotide overhangs that are homologous to genomic DNA at the insertion site. FIG. 15B depicts additional examples of DNA repair templates including sequences flanked by sgRNA target sites to generate double stranded DNA breaks flanking an inserted sequence in concert with Cas9/sgRNA cutting of the genome (top), single stranded DNA containing long homology arms flanking an inserted sequence (middle), and short homology arms flanking an inserted sequence produced by annealing of a DNA oligo (bottom).

[0032] FIG. 16. Schematic depicting: (top) a modified heavy chain locus, modified with a genetic construct encoding a heavy chain variable region of an anti-RSV antibody; (middle) a modified light chain locus, modified with a genetic construct encoding a light chain variable region of an anti-RSV antibody; and (bottom) a modified heavy chain locus, modified with a genetic construct

encoding a light chain (i.e., IgL) of an anti-RSV antibody, and a heavy chain variable region of an anti-RSV antibody, with a linker (including a Strep-Tag) between the light chain and the heavy chain variable region.

[0033] FIG. 17. Schematic of simultaneous protection against multiple pathogens by modified memory B cells and modified antibody-secreting B cells, as disclosed herein.

[0034] FIGs. 18A-18C. Efficient Cas9 cutting of a targeted intronic region in mouse and human B cells. Electroporation with Cas9/sgRNA ribonuclear protein complexes mediated effective cutting in mouse and human B cells. Cells were electroporated with Cas9/sgRNA complexes. Editing efficiency was assessed at 3 days post electroporation by Tracking of Indels by Decomposition (TIDE) in: (FIG. 18A) mouse B cell line (A20); (FIG. 18B) primary B cells; and (FIG. 18C) human B cell line (Ramos).

[0035] FIG. 19. Insertion of a genetic construct encoding an RSV-specific antibody into mouse B cells.

[0036] FIG. 20. Enrichment and analysis of genetically-modified B cells.

[0037] FIG. 21. Genetically-modified B cells secrete RSV binding antibody.

[0038] FIGs. 22A, 22B. Non-homologous end joining (NHEJ) and micro-homology mediated end joining (MMEJ) approaches offer alternatives to long-homology-directed repair (HDR) for genome engineering of primary mouse B cells. (FIG. 22A) Primary B cells were primed 24 hours, co-incubated with adeno-associated virus (AAV) for 12 hours, washed and either electroporated or transferred directly into secondary culture for 3 days before analysis of mCherry expression. (FIG. 22B) Primary B cells were primed for 24 hours, mock electroporated, or electroporated with template + Cas9/sgRNA and transferred to secondary culture for 5 days before analysis of mCherry expression.

[0039] FIGs. 23A-23E. Insertion of a novel genetic construct encoding a functional antibody into mouse and human B cell lines enables expression of surface bound and secreted antibody. (FIG. 23A) Diagram of the IgH locus showing site for insertion of partial antibody construct, as well as depictions of a surface bound and secreted antibody. emAb = synthetic antibody, herein used interchangeably with synAb. (FIG. 23B) Staining of unmodified or anti-RSV synthetic antibody (αRSV synAb) modified mouse A20 B cell lines with the RSV-prefusion-F protein tetramer (RSV-Tetramer) and anti-Streptag II tetramer (αTagAb Tetramer). (FIG. 23C) Staining of unmodified or anti-RSV synAb modified human RAMOS B cell lines with the RSV-prefusion tetramer and anti-Streptag II antibody tetramer. (FIG. 23D) ELISA for binding of antibody to RSV prefusion F protein from the culture media of unmodified or anti-RSV synAb modified A20 cell cultures. Palivizumab was used as a positive control. (FIG. 23E) ELISA for binding of antibody to RSV prefusion F

protein from the culture media of unmodified or anti-RSV synAb modified RAMOS cell cultures. Palivizumab was used as a positive control.

[0040] FIGs. 24A–24C. Production of primary mouse B cells with a novel specificity. (FIG. 24A) Surface staining of mock treated (top) or anti-RSV synAb modified mouse B cell lines (bottom) with anti-Streptag II tetramer before enrichment (left panel) and after enrichment and expansion with anti-Streptag II tetramer (middle panel) and RSV-pre-fusion viral protein tetramers (right panel). (FIG. 24B) ELISA for binding of antibody to RSV prefusion F protein from the culture media of unmodified or anti-RSV synAb modified mouse B cell cultures. Palivizumab was used as a positive control. Antibody binding detected with a 1:1 mixture of polyclonal anti-human Ig and anti-mouse Ig bound to HRP. (24C) Rapid expansion of enriched B synAb cells in culture with 3T3-CD40L feeder cells and IL-21.

[0041] FIGs. 25A-25I. Exemplary sequences. (FIG. 25A) Exemplary sgRNA sequences (SEQ ID NOs: 87, 88, 89), genome homology regions (SEQ ID NOs: 90-95), and splicing oligonucleotides (SEQ ID NOs: 96-101); (FIG. 25B) human anti-RSV-emAb AAV (2531 bp (SEQ ID NO: 102) and associated nucleotide and protein sequences (SEQ ID NOs: 110-126, 280, 285)); (FIG. 25C) mouse anti-RSV-emAb AAV (3134 bp (SEQ ID NO: 103) and associated nucleotide and protein sequences (SEQ ID NOs: 127-141, 281, 286)); (FIG. 25D) mouse emAb-RSV-dsDNA (1736 bp (SEQ ID NO: 104) and associated nucleotide and protein sequences (SEQ ID NOs: 142-144)); (FIG. 25E) human emAb-VRC01-AAV (2551 bp (SEQ ID NO: 105) and associated nucleotide and protein sequences (SEQ ID NOs: 145-152, 282, 287)); (FIG. 25F) human-emAb-Medi8852-AAV (2544 bp (SEQ ID NO: 106) and associated nucleotide and protein sequences (SEQ ID NOs: 153-160, 283, 288)); (FIG. 25G) human-emAb-AMM01-AAV (2555 bp (SEQ ID NO: 107) and associated nucleotide and protein sequences (SEQ ID NOs: 161-169, 284, 289)); (FIG. 25H) Balb/C mRSV-splice integration sequence (2261 bp (SEQ ID NO: 108) and associated nucleotide and protein sequences (SEQ ID NOs: 170-172)); and (FIG. 25I) TT-hRSV-T7-integrated sequence (1707 bp (SEQ ID NO: 109) and associated nucleotide and protein sequences (SEQ ID NOs: 173-175)).

[0042] FIGs. 26A-26D. (FIG. 26A) Targeted area upstream of the Eμ enhancer for insertion of a new antibody cassette; by targeting this region, inserted emAb genes can be driven by a native (but inserted) IgH promoter, maximizing the native control of immunoglobin expression. To enable one-hit insertion and minimize off-target interactions, emAb constructs were expressed as a single chain fusion. This fusion consists of a full light chain sequence, linked to the variable region of the heavy chain with a 57 amino acid glycine-serine linker. Physically linking the light and heavy chains minimizes the possibility of misspairing between an inserted emAb and endogenous light

chain. An optimized splice junction allows emAbs to splice to downstream endogenous IgH constant regions. This allows emAbs to be expressed as any of the heavy chain isotype classes. (FIG. 26B) The Burkitts-lymphoma derived B cell line natively expresses surface and secreted forms of IgM paired with a lambda light chain. Expression of an engineered αRSV-emAb derived from Palivizumab was detected using monomeric RSV-F protein and streptactin, a modified streptavidin with high affinity for the Streptag II motifs in the linker. αRSV-emAb modified RAMOS cells expressed the engineered RSV-specific antibody, which could be detected on the surface of cells. (FIG. 26C) The engineered RSV-specific antibody was also detected in secreted form in the supernatant. (FIG. 26D) αRSV-emAb modified cells but not control cells exhibited rapid and sustained calcium signaling in response to protein antigen.

[0043] FIGs. 27A-27G. Human B cells are efficiently genetically-modified to express single chain emAb by paired cas9-sgRNA and AAV template delivery. (FIG. 27A) Schematic representation of human cell engineering process. Day 0: B cells are isolated from PBMC and primed with CD40L, IL2, IL10, IL15, and CpG oligonucleotides. Day 2: cells are electroporated with cas9/sgRNA RNP and treated with AAV encoding the emAb HR template 1 hr post electroporation, followed by culture as described for day 0. Day 4: cells are selected on antigen binding or tag expression. Day 4-15L: selected cells are expanded on irradiated feeder cells expressing CD40L, IL2, and IL21, supplemented with IL15. Days 15-18: Cells transitioned to feeder-free differentiation culture with IL6, IL15, and IFNγ. (FIG. 27B) Indel frequency in B cells from 6 independent PBMC donors treated with emAb-targeting Cas9/sgRNA RNPs. (FIG. 27C) All human SNPs with an reported frequency across the targeting sgRNA site. (FIG. 27D) Representative FACS for binding of RSV-F prefusion monomer to control cultured or RSV-emAb genetically-modified human B cells at day 4 of culture. (FIG. 27E) Frequency of RSV-emAb B cells after engineering of B cells from 6 independent donors. (FIG. 27F) FACS for plasma cell markers (CD19, CD27, CD38 and CD138) in primed cells (Day 2) and cells differentiated in vitro (Day 18). (FIG. 27G) ELISA for secreted anti-HA-stem antibody in the culture media of control B cells or influenza targeted MEDI8852-emAb B cells at day 18 of culture.

[0044] FIG. 28. Single chain emAb is a flexible platform for expression of antiviral antibodies. Human B cells were mock electroporated (Control B, top row) or genetically-modified with emAb constructs (bottom row) derived from the variable regions of the indicated broadly neutralizing antibody constructs and human kappa (Palivizumab, VRC01, and MEDI8852) or lambda (AMM01) light chains. Control and emAb engineered cells were stained with a matching antigen derived from the indicated pathogen: RSV-F monomer, or tetramers of HIV-ENV, EBV GH/GL, or HA-stem.

[0045] FIGs. 29A-29D. emAb insertion on the productive IgH allele can block endogenous IgH production. (FIG. 29A) Diagram of RAMOS IgH alleles: one productive allele containing an emAb target site, and one allele with a c-myc translocation eliminating the emAb target site. (FIG. 29B) Flow cytometry showing surface expression of lambda light chain and RSV-F antigen binding in input RAMOS cells (gated on CD79b+), and αRSV-emAb engineered RAMOS cells (gated on CD79b+/RSV-F+). (FIG. 29C) Diagram of primary IgH alleles: one productive allele, and one non-productive allele without functional recombined VDJ, both of which contain an emAb target site. (FIG. 29D) Flow cytometry showing surface expression of λ light chain and RSV-F antigen binding on input sorted λ light chain + B cells (gated on CD79b+) and αRSV-emAb engineered B cells (gated on CD79b+/RSV-F+).

[0046] FIG. 30A-30E. Engineering of primary mouse B cells with an αRSV-emAb cassette (FIG. 30A) Schematic representation of the mouse B cell engineering process. Day 0: B cells are isolated from Spleen and peripheral lymph nodes (PLN) via negative selection and primed with CD40L-HA, anti-HA mAb, and IL4. Day 1: cells are electroporated with cas9/sgRNA RNP together with dsDNA (dsDNA condition), or cas9/sgRNA RNP alone followed by treatment with AAV containing the emAb HR template 1 hr post electroporation (AAV condition). Cells were then maintained in culture as described for day 0. Day 3: cells are selected on antigen binding or tag expression. Day 4-8L: selected cells are expanded on irradiated feeder cells expressing CD40L, supplemented with IL-21. (FIG. 30B) Indel percentage in B cells treated with IgH targeting cas9/sgRNA RNP. (FIG. 30C) Representative FACS for binding to monomeric prefusion-RSV-F protein in control B cells, or emAb B cells engineered using a dsDNA or AAV template. (FIG. 30D) Frequency of emAb cells in B cells engineered with dsDNA or AAV templates. (FIG. 30E) Anti-RSV specific secreted antibody in the supernatant of control B cells, or B cells engineered with using a dsDNA or AAV template.

[0047] FIGs. 31A-31D. Protection from viral infection by engineered αRSV-emAb B cells. (FIG. 31A) Schematic representation of antiviral protection by transferred emAb cells. Day 0: $1.5x10^7$ enriched RSV-emAb B cells are transferred via I.P. injection. Day 5: Palivizumab I.P. injection at 15 mg/kg. Day 6: Blood draw to measure antiviral Ab titers. Day 7: intranasal challenge with 10^6 pfu RSV virus. Day 12: measurement of viral titer in lungs. (FIG. 31B) Surface expression of RSV-emAb receptor before or 24 hours after transfer of RSV-emAb cells measured with RSV-F monomer and streptactin tag binding. (FIG. 31C) Plasma titer of αRSV-F antibodies in mice at day 6. (FIG. 31D) Viral titers of RSV in the lungs of mice with no cells transferred, with αRSV-emAb B cells, with control B cells, or with 15 mg/kg Palivizumab delivered I.P. 48 hours prior to infection.

[0048] FIGs. 32A, 32B. Mutiplex transfer of human antibody secreting cells to NSG mice (FIG. 32A) Schematic of transfer of human emAb B cells into NSG mice. Day 0: 5×10^6 anti-Flu emAb B cells and 5×10^6 anti-RSV emAb B produced as described in FIG. 27 were transferred via I.P. injection. Day 7: Blood draw for antibody production in serum. (FIG. 32B) ELISA for serum titers of anti-RSV-F and anti-HA-stem antibodies in mice which received emAb cells (Dual transfer) versus control serum (no transfer).

[0049] FIGs. 33A, 33B. Templates for long insertion of emAb cassettes into the mouse (FIG. 33A) and human (FIG. 33B) IgH locus. Indicated on the top row for each genome are the position of elements in germline IgH loci, including the final J regions, the Eμ intronic enhancer element, and the beginning of the μ constant domain. The position of the cas9/sgRNA target site is indicated (Cut site). Below is shown the positions of the targeting arms targeting homology arms included in the mouse AAV and dsDNA construct (FIG. 33A) as well as the human AAV construct (FIG. 33B). Also shown is the emAb cassette as inserted in the genome.

DETAILED DESCRIPTION

[0050] Vaccines are designed to increase the immunity of a subject against a particular infection by stimulating B cells to produce antibodies against the targeted infectious agent. Antibodies are proteins that can provide protection against pathogens. Antibodies can bind to a pathogen and are protective when this binding interferes with the normal function of a pathogen. For example, many protective antibodies bind to a portion of a pathogen that blocks the pathogen from entering cells. Antibodies can be attached to the surface of B cells (known as B cell receptors), but exert most of their protective functions when secreted into the blood.

[0051] Pathogen can refer to any substance that can cause disease, and pathogenic can refer to the ability of a substance to cause disease. Examples of pathogens include viruses, bacteria, and fungi that can infect a host and cause disease. Other examples of pathogens include host-derived proteins or other host-derived substances that cause disease, such as tumor necrosis factor alpha (TNFα), an inflammatory molecule associated with numerous autoimmune conditions (e.g., arthritis) and beta amyloid plaques, which are fibrous proteins that accumulate in the brain during Alzheimer's disease. In particular embodiments, cancer cells and/or tumors can also be referred to as pathogens or pathogenic substances, based on their ability to cause disease.

[0052] Upon exposure to a vaccine or a natural pathogen, an epitope provided in the vaccine and/or present on the pathogen can bind to a B cell receptor present on a naïve B cell. This binding can lead to activation of the B cell and production of protective antibodies.

[0053] A naïve B cell refers to a B cell before it has come in contact with its epitope. Each naïve

B cell expresses a unique antibody with unique epitope specificity. The unique antibody expressed by each naïve B cell is generated randomly through genetic recombination. Naïve B cells express membrane-bound antibodies (i.e., B cell receptors) and upon epitope binding, can rapidly proliferate. During proliferation and maturation, the antibody genes undergo somatic mutation, which serves to increase the affinity of epitope binding. The increase in affinity of epitope binding that occurs during B cell maturation is required for effective protection against the pathogen. A single naïve B cell is able to undergo dozens of cell divisions to create thousands of antibody-secreting B cells and memory B cells (FIG. 1) expressing the same antibody, or a related antibody that has been mutated to improve binding to the pathogen.

[0054] In addition to active antibody-secreting B cells, memory B cells are important for protection against pathogens. Memory B cells do not normally actively secrete antibodies but can rapidly differentiate into antibody-secreting cells. The rapid differentiation of memory B cells into antibody-secreting cells can help the immune system mount a rapid response to a secondary infection or a pathogen that has previously been encountered through vaccination (McHeyzer-Williams et al., Nat Rev Immunol. 2011;12(1):24-34; Taylor et al., Trends Immunol. 2012;33(12):590-7). For example, memory B cells maintain protection against Hepatitis B virus when the level of antibody produced by antibody-secreting B cells has diminished (Williams et al., Vaccine. 2001;19(28-29):4081-5; Bauer et al., Vaccine. 2006;24(5):572-7). Thus, successful vaccines stimulate the generation of antibody-secreting B cells and long-lived memory B cells, all capable of expressing antibodies that bind to an epitope on the pathogen with high affinity.

[0055] Unfortunately, there are many infectious agents for which no vaccines are available. Examples of infectious agents without an available effective vaccine strategy include RSV, HIV, and Zika virus.

[0056] Regarding RSV, the disastrous failure of a formalin-inactivated RSV vaccine in the 1960s was likely not due to a failure to induce antibody-secreting B cells and memory B cells targeting RSV. It is likely that the vaccine induced the production of antibodies that did not neutralize RSV, but instead enhanced RSV infection (FIG. 2A) (Blanco et al., Hum Vaccin. 2010;6(6):482-92; Broadbent et al., Influenza Other Respir Viruses. 2015;9(4):169-78). This highlights the delicate balance that must be achieved by vaccines: induction of the production of "protective" antibodies targeting certain epitopes while avoiding stimulating the production of "pathogenic" antibodies targeting the incorrect epitopes (FIG. 2A).

[0057] A 2015 analysis of the World Health Organization International Clinical Trials Registry Platform identified nine candidate RSV vaccines evaluated clinically since 2008, none of which progressed beyond Phase 2 of testing (Broadbent et al., Influenza Other Respir Viruses.

2015;9(4):169-78). Amongst these, only three trials have been completed and only one has reported results. That vaccine, called MEDI-559, appears to reduce RSV infection, but respiratory symptoms were too high for further testing (Malkin et al., PLoS One. 2013;8(10):e77104). These data suggest that while MEDI-559 induced the production of protective antibodies, it likely also induced the production of pathogenic antibodies (FIG. 2B).

[0058] Other "improved" vaccination strategies involve changing the formulation that is administered to patients. These include alternative methods to inactivate/attenuate the virus, and changes to the adjuvant aimed at increasing the inflammatory response (Broadbent et al., Influenza Other Respir Viruses. 2015;9(4):169-78; Garg et al., The Journal of general virology. 2014;95(Pt 5):1043-54; Swanson et al., J Virol. 2014;88(20):11802-10; Widjaja et al., PLoS One. 2015;10(6):e0130829; Stewart-Jones et al., PLoS One. 2015;10(6):e0128779). Some of these approaches have yielded increases in protective antibodies in animal models, but the possibility of inducing pathogenic antibodies makes it likely that these "improved" RSV vaccines would suffer the same fate as MEDI-559.

[0059] In an effort to focus the immune response to the epitopes targeted by protective antibodies, a recent approach has been to graft a single RSV epitope onto a non-RSV scaffold (FIG. 2C). This approach eliminates the possibility of pathogenic antibodies specific for other RSV epitopes since they are absent from the scaffold. RSV epitope scaffold vaccination of rhesus macaque resulted in the production of neutralizing antibodies by some animals, but only after 3-5 injections (Correia et al., Nature. 2014;507(7491):201-6).

[0060] Approaches to bypass vaccination and directly provide protective antibodies have also been developed. The only clinically approved prophylactic treatment for RSV is the injection of the high affinity RSV-specific protective antibody Palivizumab (FIG. 3A) (The PREVENT Study Group. Pediatrics. 1997;99(1):93-9; The IMpact-RSV Study Group. Pediatrics. 1998;102(3 Pt 1):531-7). Unfortunately, the $10,000 cost of a 5-month series of Palivizumab has limited its use to children at high-risk for severe RSV infection (Meissner & Kimberlin, Pediatrics. 2013;132(5):915-8). Other RSV-specific antibodies designed to last up to a year are currently undergoing clinical evaluation (Influenza Other Respir Viruses. 2015;9(4):169-78). However, yearly antibody re-injection is not feasible for life-time protection.

[0061] To eliminate the need for life-long injections, methods have been developed in which an adenoviral vector is used to transfer a gene encoding a protective antibody into muscle cells (FIG. 3B) (Schnepp & Johnson, Curr Opin HIV AIDS. 2014;9(3):250-6). Promisingly, adenovirus-mediated expression of Palivizumab partially protected mice from RSV infection (Skaricic et al., Virology. 2008;378(1):79-85). A limitation of this approach, however, is the high costs of

manufacturing the high doses of virus necessary to achieve protective levels of antibody (24). The high doses are necessary because the expression of antibody by a muscle cell is low compared to the estimated 10,000 antibodies secreted per second by a single B cell (Helmreich et al., J Biol Chem. 1961;236:464-73; Hibi & Dosch, Eur J Immunol. 1986;16(2):139-45). B cells achieve this high rate of secretion by completely reprogramming their protein production machinery to focus upon antibody secretion. Without a revolution in manufacturing capability, adenovirus-mediated antibody gene transfer into muscle cells is not a realistic option for RSV prevention.

[0062] Another approach uses a lentiviral vector to incorporate the genes encoding protective antibodies into the genome of hematopoietic stem cells, which would be subsequently induced to differentiate into antibody-secreting B cells (FIG. 3C). One limitation of this approach is that antibody gene insertion is random, which introduces the risk of off-target genetic effects resulting in disease. A second limitation of this approach is the long two-month in vitro culture conditions necessary to induce differentiation of antibody-secreting cells from hematopoietic stem cells (Luo et al., Blood. 2009;113(7):1422-31). A final limitation is that this strategy will not generate a source of antibody that can be augmented upon infection. Therefore, if the antibody-secreting cells are not present in high numbers, or are not long-lived, protection against infection will be inadequate

[0063] In particular embodiments, the current disclosure provides bypassing vaccination and/or eliminating the need for repeated therapeutic antibody injections by genetically engineering B cells to express a selected antibody (e.g., an antibody against an infectious agent (e.g., palivizumab; FIG. 4A)). Types of B cells that are particularly useful to genetically engineer include existing antibody-secreting B cells, memory B cells, naïve B cells, B1 B cells, and marginal zone B cells. Naive B cells have the greatest proliferative and functional potential and can enter the germinal center response and improve their binding ability. B1 B cells express BCR and migrate to different locations such as the peritoneal cavity. B1 B cells rapidly differentiate into antibody-secreting cells upon stimulation through the BCR, and do not require signals for T cells for optimal function. Marginal zone B cells are largely located in the marginal zone of the spleen and rapidly differentiate into antibody-secreting cells upon stimulation through the BCR. Marginal zone B cells also do not require signals from T cells for optimal function. Genetically engineering one or more of these subsets of B cells can create a baseline level of antibody to treat ongoing or immediate infection, and a long-lived source of inducible antibody in case of future re-infection. FIG. 4B demonstrates an associated strategy for simultaneous protection against numerous pathogens utilizing the teachings of the current disclosure while FIG. 5 depicts hypothesized secretion of palivizumab by exemplary B cell subtypes in the presence and absence of infection.

[0064] The current disclosure provides genetic engineering of B cells by inserting a genetic

construct including a transgene into an endogenous antibody gene locus specifically chosen to take advantage of the structure and function of the endogenous B cell genome. For example, inserting a transgene encoding at least a portion of a selected antibody into an endogenous antibody gene locus can allow for robust production of the selected antibody by taking advantage of the endogenous antibody expression regulatory machinery. Transgene may refer to a section of DNA that encodes a foreign (i.e., exogenous) protein. Genetic construct may refer to an artificially constructed segment of nucleic acid which is intended for introduction into a cell to allow expression of the foreign protein.

[0065] In particular embodiments, the current disclosure provides B cells that are modified to express a selected antibody. Antibodies are produced from two genes, a heavy chain gene and a light chain gene. Generally, an antibody includes two identical copies of a heavy chain, and two identical copies of a light chain (see, e.g., FIG. 6B). The heavy chains are the larger subunits of the two and each heavy chain includes a VDJ segment and a constant region (shown as "C" in FIG. 6B). The VDJ segment (or VDJ) refers to the unique pairing of V, D and J gene segments that encode the unique portion of the antibody heavy chains that bind to an epitope on a pathogen. Thus, V refers to one of the gene segments that randomly pairs with a D and J segment to encode the unique portion of the antibody heavy chain that binds to an epitope on the pathogen. Similarly, D refers to one of the gene segments that randomly pairs with a V and J segment to encode the unique portion of the antibody heavy chain that binds to an epitope on the pathogen. Finally, J refers to one of the gene segments that randomly pairs with a V and D segment to encode the unique portion of the antibody heavy chain that binds to an epitope on the pathogen. There are several V segments, D segments, and J segments that can come together in a variety of distinct combinations to form the particular VDJ segment of a particular heavy chain (see, e.g. FIG. 7).

[0066] Each B cell pairs just a single VDJ combination with a conserved constant, C, region to form a full-length heavy chain. The heavy chain C region can interact with other immune proteins, such as Fc receptors to activate other immune cells. All naive B cells express the same C region segments, but can change to express different C region segments following activation, and different C regions give antibodies different functions. For example, one C gene segment encodes ε, and antibodies expressing ε are "IgE". IgE-type antibodies bind to cells of the body and often mediate allergic reactions. Antibodies expressing an α C region are IgA antibodies; antibodies expressing a γ C region are IgG antibodies and antibodies expressing a μ C region are IgM antibodies. The human genome includes a single heavy chain locus, which is present on chromosome 14.

[0067] Again referring to FIG. 6B, the light chain of an antibody (IgL) includes a variable region

and a constant region. The light chain variable region includes V and J gene segments, and the light chain constant region can include a single immunoglobulin constant domain. Humans express two different light chains: Igκ, which is encoded by the immunoglobulin kappa locus on chromosome 2; and Igλ, which is encoded by the immunoglobulin lambda locus on chromosome 22.

[0068] FIG. 6A depicts schematics of the endogenous B cell genome that encode an IgH chain and an IgL chain. FIGs. 8A and 8B depict initial schematics of where exogenous genetic constructs according to the current disclosure can be inserted to achieve expression of a selected antibody. FIG. 8A depicts inserting a genetic construct including [a stop signal, an IgL chain of a selected antibody (here, PV), a skipping element (here, 2A), and the VDJ segment of a heavy chain] into the endogenous IgH genome between the endogenous VDJ segment and the endogenous C region coding segments. This approach leads to expression of an entire exogenous IgL chain, an exogenous VDJ segment of a heavy chain, and an endogenous C region of the heavy chain. Expression of an antibody that includes an endogenous C region can be useful, for example, because it can allow a modified B cell to modulate C region expression based on natural B cell activation and maturation state. For example, alternative splicing in the constant region of the heavy chain gene locus can allow a modified B cell to switch between expression of a membrane-bound antibody and expression of a secreted antibody. This approach also allows expression of an exogenous VDJ without requiring excision of the endogenous VDJ. This feature is beneficial because the VDJ is a relatively large segment of DNA and its excision can negatively affect cellular function.

[0069] FIG. 9 depicts a similar schematic with more detail regarding the structure and function of the endogenous B cell genome, and how the current disclosure utilizes this structure and function to achieve expression of selected antibodies. Promoter regions are necessary to achieve transcription of a gene segment. Heavy chain variable region (V_H) promoters are selectively active in the B cell lineage, and include a TATA box, an Inr element, and an octamer element within 100 base pair (bp) of the transcriptional initiation site. V_H promoter activity is under proximity-dependent regulation by the endogenous B cell genome's Eμ enhancer element (gray oval), and an enhancer element positioned at the 3' end of the heavy chain gene locus, proximal to the heavy chain α constant gene (gray circle). The Eμ enhancer element is an intronic region of DNA (40 to 1500 bp in length) within the 700-bp intron between the J heavy chain segment and the C mu (μ) segment of the immunoglobulin heavy chain gene locus. It can bind an activator protein to increase or activate transcription of the heavy chain gene. The sequence of the human Eμ enhancer element is provided in FIG. 11A as SEQ ID NO: 85. The sequence of the mouse Eμ

enhancer element is provided in FIG. 13A as SEQ ID NO: 86.

[0070] Inserting a genetic construct that includes a V_H promoter between an endogenous heavy chain variable region and an endogenous Eμ enhancer may reduce or block transcription activation from the endogenous V_H promoter because the Eμ enhancer will initiate transcription at the most proximal upstream promoter. In this manner, expression of the endogenous VDJ can be blocked without requiring the removal of such a large DNA segment (which, as indicated, is problematic for cell function and survival). In particular embodiments, VDJ recombination removes genetic material between the V_H promoter and the Eμ enhancer, which positions the enhancer at the appropriate distance from an exogenous promoter of a genetic construct disclosed herein to activate transcription starting from the promoter in the inserted genetic construct. In particular embodiments, no endogenous genetic material is removed. In particular embodiments, less than 50 base pairs are removed. In particular embodiments, V_H promoters within exogenous genetic constructs include the native light chain promoter for IgK or IgL, a native human IgH promoter, the spleen focus forming viral promoter SFFV, the J558 h10 promoter or the IgVH1-69 promoter.

[0071] FIG. 10 provides an additional schematic for target areas for genetic construct insertion. These target areas encompass two conserved regions present in all B cells: from the terminal J gene segment (IGHJ6 in humans, IGHJ4 in mice) to the heavy chain intronic enhancer (Eμ), and from Eμ to the repetitive sequences associated with DNA switch recombination.

[0072] In particular embodiments, the area of the endogenous B cell genome that is targeted for insertion of the genetic construct is upstream of the Eμ enhancer of SEQ ID NOs: 85 or 86. FIGs. 11A-14B provide particular sequences that can be targeted for genetic construct insertion to achieve expression of a selected antibody as disclosed herein.

[0073] FIG. 11A provides the human DNA sequence of IGHJ6 to Eμ intronic enhancer (SEQ ID NO: 1; >hg38_dna range=chr14:105862523-105863244 5'pad=0 3'pad=0 strand=- repeatMasking=none). FIG. 11B provides exemplary ranges to target (e.g., gRNA sites) within this sequence including SEQ ID NOs: 5-24 and associated gRNA sequences (SEQ ID NOs: 88, 89, and 290-307). As examples, in particular embodiments, sgRNA of SEQ ID NO: 88 (see also FIG. 25A) can be used to target gRNA site of SEQ ID NO: 7. In particular embodiments, sgRNA of SEQ ID NO: 89 (see also FIG. 25A) can be used to target gRNA site of SEQ ID NO: 10.

[0074] FIG. 12A provides the human DNA sequence for Region 2: Eμ intronic enhancer to switch region (SEQ ID NO: 2; >hg38_dna range=chr14:105860383-105861690 5'pad=0 3'pad=0 strand=-). FIG. 12B provides exemplary ranges to target (e.g., gRNA sites) within this sequence including SEQ ID NOs: 25-44 and associated gRNA sequences (SEQ ID NOs: 308-327).

[0075] FIG. 13A provides the mouse DNA sequence for region 1: from IGHJ4 to Eμ intronic

enhancer (SEQ ID NO: 3; >mm10_dna range=chr12:113427973-113428554 5'pad=0 3'pad=0 strand=- repeatMasking=none). FIG. 13B provides exemplary ranges to target (e.g., gRNA sites) within this sequence including SEQ ID NOs: 45-64 and associated gRNA sequences (SEQ ID NOs: 87, and 328-346). As an example, in particular embodiments, sgRNA of SEQ ID NO: 87 (see also FIG. 25A) can be used to target gRNA site of SEQ ID NO: 46.

[0076] FIG. 14A provides the Mouse DNA sequence for region 2: from Eμ intronic enhancer to switch region (SEQ ID NO: 4; >mm10_dna range=chr12:113425446-113426973 5'pad=0 3'pad=0 strand=- repeatMasking=none). FIG. 14B provides exemplary ranges to target (e.g., gRNA sites) within this sequence including SEQ ID NOs. 65-84 and associated gRNA sequences (SEQ ID NOs: 347-366).

[0077] Thus, in particular embodiments, the current disclosure provides targeted insertion of a genetic construct including (i) a promoter and (ii) a transgene encoding a portion of a selected antibody at an intronic region that is constant in all B cells (before and after recombination) and (i) positioned relative to an enhancer element that interacts with the promoter; and (ii) in a configuration such that the B cells' endogenous heavy chain VDJ sequence is not expressed. In particular embodiments, the encoded portion of the selected antibody includes the entire light chain of the antibody and the VDJ segment of the heavy chain. These portions of the selected antibody can be expressed with a heavy chain constant region expressed by the modified B cell at any given time. Particular embodiments of the genetic construct may also include or encode a signal peptide, a flexible linker, a skipping element, and or a splice junction.

[0078] One technical challenge of the current disclosure is that an antibody is a protein made from two separate gene products, the heavy chain (IgH) and the light chain (IgL) (FIGs. 6A, 6B). This means that, in particular embodiments, both genetic locations must be simultaneously modified in order to properly express a selected antibody. However, the current disclosure also provides strategies to produce functional selected antibodies without necessitating modifying both genetic locations. One approach that allows this is through the use of sequences that allow antibody expression through a single construct. In particular embodiments, this is achieved by including a skipping element within the genetic construct. One example of a skipping element is a self-cleaving peptide, such as a self-cleaving "2A" peptide. 2A peptides function by causing the ribosome to skip the synthesis of a peptide bond at a defined location, leading to production of two proteins from one mRNA. The 2A sequences are short (e.g., 20 amino acids), facilitating use in size-limited constructs, and proteins are produced at a 1:1 ratio. Particular examples include T2A (GSG)EGRGSLLTCGDVEENPGP (SEQ ID NO: 176); P2A (GSG)ATNFSLLKQAGDVEENPGP (SEQ ID NO: 177); E2A (GSG)QCTNYALLKLAGDVES

NPGPP (SEQ ID NO: 178); and F2A (GSG)VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 179).

[0079] In particular embodiments, the genetic constructs include an internal ribosome entry site (IRES) sequence. The IRES can be positioned upstream of the heavy chain VDJ of the genetic construct. IRES are non-coding structured RNA sequences that allow ribosomes to initiate translation at a second internal site on a mRNA molecule, leading to production of two proteins from one mRNA. However, IRES driven translation is less efficient than 2A driven translation, leading to lower expression of the second protein in the transcript.

[0080] In particular embodiments, the genetic constructs encode a flexible linker between the light chain portion of the selected antibody and the heavy chain portion of the selected antibody. A linker can be a series of amino acids that flexibly link one protein domain to another protein domain in a way that allows the linked sequences to interact to form a functional unit.

[0081] In particular sequences, flexible linkers can include one or more series of combinations of glycine and serine, which provide flexibility to the linker sequence. Exemplary Gly-Ser linkers include (GGS)n (SEQ ID NO: 180), (GGGS)n (SEQ ID NO: 181), and (GGGGS)n (SEQ ID NO: 182) wherein n = 1 – 100 and every integer therebetween. In particular embodiments, n = 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30. In particular embodiments, a Gly-Ser linker includes 50-80 amino acids. In particular embodiments, the Gly-Ser linker includes 54, 57, or 60 amino acids. In particular embodiments, the Gly-Ser linker is encoded by SEQ ID NO: 116. In particular embodiments, the Gly-Ser linker includes SEQ ID NO: 122.

[0082] Additional examples of flexible linkers include (KESGSVSSEQLAQFRSLD)n (SEQ ID NO: 183) and (EGKSSGSGSESKST)n (SEQ ID NO: 184). In these linkers the Gly and Ser residues in the linker were designed to provide flexibility, whereas Glu and Lys were added to improve the solubility. Bird, RE et al. Science, 1988;242:423–426. In particular embodiments, n = 1 – 100 and every integer therebetween. In particular embodiments, n = 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30. In particular embodiments, these linkers includes 50-80 amino acids.

[0083] Particular embodiments include a splice junction that allows splicing between RNA encoded by the genetic construct and RNA encoded by the endogenous heavy chain constant region. In particular embodiments, the genetic constructs include a splice junction sequence at the 3' end. Splicing can refer to the removal of introns and joining together of exons by an RNA/protein complex known as the spliceosome. A splice junction refers to an intronic sequence directly flanking an exon. A splice junction at the 3' end of an exon can include a splice donor site. Splice donor site sequences typically begin with "GU". In particular embodiments, the splice

junction may include 40-80 bp of an intron following the last exon of a VDJ. In particular embodiments, the splice junction includes 40-80 bp of the intron flaking the 3' end of the human IGHJ1 gene segment or the mouse IGHJ3 gene. In particular embodiments, the splice junction includes CAG/gtaagt, with the cut and splice taking place after the uppercase G (indicated by the "splice" annotation). In particular embodiments, the splice junction includes CAG/gtgagt. The CA form the end of a serine codon, and the G begins the first codon from the constant region. In particular embodiments, a splice junction with flanking sequence includes SEQ ID NOs: 124 or 151 in genetic constructs for insertion into a human locus. In particular embodiments, a splice junction with flanking sequence includes SEQ ID NO: 139 in genetic constructs for insertion into a mouse locus.

[0084] Genetic constructs disclosed herein can also encode signal peptides. Exemplary signal peptides include signal peptides derived from human IgH heavy chains, such as MELGLSWIFLLAILKGVQC (SEQ ID NO: 185); MELGLRWVFLVAILEGVQC (SEQ ID NO: 186);MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 187); MDWTWRILFLVAAATGAHS (SEQ ID NO: 188); MDWTWRFLFVVAAATGVQS (SEQ ID NO: 189); MEFGLSWLFLVAILKGVQC (SEQ ID NO: 190); MEFGLSWVFLVALFRGVQC (SEQ ID NO: 191); and MDLLHKNMKHLWFFLLLVAAPRWVLS (SEQ ID NO: 192); and signal peptides derived from human IgL light chains, such as MDMRVPAQLLGLLLLWLSGARC (SEQ ID NO: 193); and MKYLLPTAAAGLLLLAAQPAMA (SEQ ID NO: 194). In particular embodiments, a signal peptide is encoded by SEQ ID NO: 112 and includes SEQ ID NO: 118 in genetic constructs for insertion into a human locus. In particular embodiments, a signal peptide is encoded by SEQ ID NO: 129 and includes SEQ ID NO: 134 in genetic constructs for insertion into a mouse locus. See also FIGs. 25B-25I and Haryadi R et al., tPLoS One v.10(2); 2015 PMC4338144.

[0085] As indicated, particular embodiments of the disclosure utilize insertion of exogenous genetic constructs at a targeted location within the endogenous B cell genome. In particular embodiments, such targeted insertion can be facilitated by including homology regions on one or both ends of the genetic construct. Homology regions (i.e., homology stitches or homology arms) are homologous to sequences at a desired insertion site. In particular embodiments, homology arms refer to segments of DNA included in a genetic construct that are 100% identical to a region of DNA that is being modified. In particular embodiments, 100% identity may not be required to achieve targeted insertion (e.g., at least 90% identity may be sufficient).

[0086] Homology regions cause the genetic construct to align next to the targeted genetic region, and portions of DNA from the genetic construct are swapped into the region cut by gene editing techniques. In particular embodiments, a genetic construct may include an upstream genome

homology end with 20 to 1,500 bp of genome homology, and a downstream genome homology end with 20 to 1,500 bp of genome homology. The regions of homology may, for example, provide "homology stitches" as shown in FIG. 15A, which can mediate insertion of the genetic construct into the targeted insertion site. In particular embodiments, the upstream genome homology end and the downstream genome homology end may include sequences with homology to genome sequences between a heavy chain VDJ region and a heavy chain Eμ enhancer element. In particular embodiments, regions of homology may particularly include 20-50 base pairs; 300-500 base pairs; 350-550 base pairs; 900-1,000 base pairs, or 400-600 base pairs. In particular embodiments, regions of homology may particularly include 30-40 base pairs (e.g., 36 base pairs); 445-455 base pairs (e.g., 450 base pairs); 495-510 base pairs (e.g., 503 base pairs); and/or 960-980 base pairs (e.g., 968 base pairs). In particular embodiments, homology regions for use in mouse genetic constructs include SEQ ID NOs: 90, 91, 96, 97, 127, 140, 142, 143, 170, and 171. In particular embodiments, homology regions for use in human genetic constructs include SEQ ID NOs: 92-95, 98-101, 110, 125, 153, 173, and 174.

[0087] In particular embodiments, the genetic constructs also encode a tag sequence. Tag sequences may be useful, for example, so that cells expressing the genetic construct may be identified and/or sorted during genetic modification processes and/or so that they can be controlled following administration to a subject. For example, in particular embodiments, it may be useful to track and/or terminate genetically modified cells following administration to a subject. Exemplary tags include STREPTAG® (GmbH, LLC, Gottingen, DE), STREP® tag II (WSHPQFEK (SEQ ID NO: 195)), or any variant thereof; *see, e.g.,* U.S. Patent No. 7,981,632), His tag, Flag tag (DYKDDDDK (SEQ ID NO:196)), Xpress tag (DLYDDDDK (SEQ ID NO: 197)), Avi tag (GLNDIFEAQKIEWHE (SEQ ID NO: 198)), Calmodulin tag (KRRWKKNFIAVSAANRFKKISSSGAL (SEQ ID NO: 199)), Polyglutamate tag, HA tag (YPYDVPDYA (SEQ ID NO: 200)), Myc tag (EQKLISEEDL (SEQ ID NO: 201)), Nus tag, S tag, SBP tag, Softag 1 (SLAELLNAGLGGS (SEQ ID NO: 202)), Softag 3 (TQDPSRVG (SEQ ID NO: 203)), and V5 tag (GKPIPNPLLGLDST (SEQ ID NO: 204)).

[0088] In particular embodiments, the current disclosure provides a genetic construct for selected antibody expression including or encoding (i) a heavy chain promoter, and/or (ii) an immunoglobulin light chain, and/or (iii) a heavy chain variable region, and/or (iv) a stop codon; and/or (v) a skipping element and/or (vi) a splice junction and/or (vii) homology arms and/or (viii) a linker and/or (ix) a tag.

[0089] Particular embodiments include or encode: (i) a heavy chain promoter; (ii) a signal peptide; (iii) an entire light chain of a selected antibody; (iv) a flexible linker or a skipping element; (v) the

variable region of a selected antibody heavy chain; and (vi) a splice junction.

[0090] Particular embodiments include or encode: (i) a heavy chain promoter; (ii) a signal peptide; (iii) an entire light chain of a selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of a selected antibody heavy chain; (vi) a splice junction, and (vii) homology arms.

[0091] Particular embodiments include or encode: (i) a heavy chain promoter; (ii) a signal peptide; (iii) an entire light chain of a selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of a selected antibody heavy chain; (vi) a splice junction, (vii) homology arms; and (viii) a tag.

[0092] FIG. 15B depicts additional examples of DNA repair templates. Examples of DNA repair templates that can also be used include synthetic DNA templates and adeno-associated viruses. In particular embodiments, synthetic DNA templates can include double stranded DNA (dsDNA) including or encoding a promoter and selected antibody portion flanked by 20-1,500 base pairs of homology to the target site in the genome. In particular embodiments, synthetic DNA templates can include single stranded DNA (ssDNA) including or encoding a promoter and selected antibody portion flanked by 10-80 base pairs, or 400-1000 base pairs of homology to the target site in the genome. In particular embodiments, synthetic DNA templates can include both dsDNA and ssDNA, terminally modified by phosphorylation to increase DNA ligation efficiency. In particular embodiments, both dsDNA and ssDNA can be terminally modified with phosphorothioate bonds to increase stability and prevent endonuclease digestion.

[0093] In particular embodiments, an adeno-associated virus can include a segment encoding a synthetic antibody portion flanked by 20-1,500 base pairs of homology to the target site in the genome. In particular embodiments, the promoter and synthetic antibody portion encoding sequence can be flanked by matching homology sequences to the target site in the genome.

[0094] In particular embodiments, the genetic construct including a DNA repair mechanism (e.g., homology stitches, synthetic DNA template) may be delivered utilizing a gene editing system, such as CRISPR, TALENs, megaTALs, zinc finger nucleases and/or an adeno-associated virus as described in more detail below. For example, a genome targeting element, a genome cutting element, and a genetic construct described herein can be administered to a B cell.

[0095] As a particular example of an application of the current disclosure, B cells may be modified to express the palivizumab antibody. The B cells may be modified with a genetic construct that includes 80 bp homology arms flanking a heavy chain promoter upstream of the complete light chain (IgLPV) and VDJ heavy chain gene segments (VDJPV) from palivizumab separated by a 2A peptide. Here, the 2A peptide is included in order to induce a ribosomal skipping event (Donnelly et al., The Journal of general virology. 2001;82(Pt 5):1013-25), which allows for the

heavy chain and light chain to be produced as separate subunits that will associate normally to form the selected antibody. In particular embodiments, a stop codon can be included upstream of the inserted heavy chain promoter to halt any potential transcription of the endogenous heavy chain variable region.

[0096] FIG. 16 depicts an example of an "as-modified" B cell genome, while FIG. 17 depicts resulting B cell populations expressing selected antibodies.

[0097] The following paragraphs provide a more detailed description regarding (i) Exemplary Selected Antibodies and Sequences; (ii) Gene Editing Techniques and Cell Sorting; (iii) Formulation of Modified B cells; and (iv) Methods of Use.

[0098] (i) Exemplary Selected Antibodies and Sequences. In particular embodiments, a selected antibody is an antibody that can provide a protective effect against a pathogen or condition (e.g., autoimmune disease). In particular embodiments, the selected antibody is an anti-RSV antibody, an anti-HIV antibody, an anti-Dengue virus antibody, an anti-*Bordatella pertussis* antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-*Clostridium difficile* bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.

[0099] In particular embodiments, the selected antibodies are chimeric antibodies. In particular embodiments, chimeric antibodies refer to a synthetic antibody that includes: (i) at least one portion that is encoded by a B cell's endogenous genome, and (ii) at least one portion that is encoded by an inserted genetic construct. In particular embodiments, the chimeric antibody includes an endogenous heavy chain constant domain, an exogenous immunoglobulin variable and constant light chain, and an exogenous variable heavy chain.

[0100] The following antibodies and sequences are useful to provide selected antibodies with targeted binding against pathogens or antigens of interest (unless noted, Kabat numbering is intended):

[0101] An exemplary anti-RSV antibody is palivizumab, which targets the RSV fusion protein and is used to prevent or reduce RSV infections.

[0102] In particular embodiments, an anti-RSV antibody is mouse palivizumab that includes a variable heavy chain sequence including:
QVELQESGPGILQPSQTLSLTCSFSGFSLSTSGMSVGWIRQPSGEGLEWLADIWWDDKKDYN
PSLKSRLTISKDTSSNQVFLKITGVDTADTATYYCARSMITNWYFDVWGAGTTVTVSS (SEQ ID NO: 138); and a variable light chain sequence including:
DIQLTQSPAIMSASPGEKVTMTCSASSSVGYMHWYQQKLSTSPKLQIYDTSKLASGVPGRFSG

SGSGNSYSLTISSIQAEDVATYYCFRGSGYPFTFGQGTKLEIK (SEQ ID NO: 205).

[0103] An additional exemplary anti-RSV antibody is human palivizumab and includes a variable light chain sequence including:

DIQMTQSPSTLSASVGDRVTITCKCQLSVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRFSG SGSGTEFTLTISSLQPDFATYYCFQGSGYPFTFGGGTKLEIKR (SEQ ID NO: 206); and a variable heavy chain sequence including:

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGWIRQPPGKALEWLADIWWDDKKDYN PSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWYFDVWGAGTT (SEQ ID NO: 123).

[0104] Within a variable heavy chain and variable light chain, segments referred to as complementary determining regions (CDRs) dictate epitope binding. Each heavy chain has three CDRs (i.e., CDRH1, CDRH2, and CDRH3) and each light chain has three CDRs (i.e., CDRL1, CDRL2, and CDRL3)

[0105] An additional exemplary anti-RSV antibody is described in U.S. Patent No. 9,403,900. This anti-RSV antibody includes a variable heavy chain including a CDRH1 sequence including GASINSDNYYWT (SEQ ID NO: 207), a CDRH2 sequence including HISYTGNTYYTPSLKS (SEQ ID NO: 208), and a CDRH3 sequence including CGAYVLISNCGWFDS (SEQ ID NO: 209); and a variable light chain including a CDRL1 sequence including QASQDISTYLN (SEQ ID NO: 210), a CDRL2 sequence including GASNLET (SEQ ID NO: 211), and a CDRL3 sequence including QQYQYLPYT (SEQ ID NO: 212).

[0106] Exemplary anti-RSV antibodies also include AB1128 (available from MILLIPORE) and ab20745 (available from ABCAM).

[0107] An example of an anti-HIV antibody is 10E8, which is a broadly neutralizing antibody that binds to gp41. The 10E8 anti-HIV antibody includes a variable heavy chain including a CDRH1 sequence including GFDFDNAW (SEQ ID NO: 213), a CDRH2 sequence including ITGPGEGWSV (SEQ ID NO: 214), and a CDRH3 sequence including TGKYYDFWSGYPPGEEYFQD (SEQ ID NO: 215); and a variable light chain including a CDRL1 sequence including TGDSLRSHYAS (SEQ ID NO: 216), a CDRL2 sequence including GKNNRPS (SEQ ID NO: 217), and a CDRL3 sequence including SSRDKSGSRLSV (SEQ ID NO: 218).

[0108] An additional example of an anti-HIV antibody is VRC01, which is a broadly neutralizing antibody that binds to the CD4 binding site of gp120. The VRC01 antibody includes a variable heavy chain including a CDRH1 sequence including GYEFIDCT (SEQ ID NO: 219), a CDRH2 sequence including KPRGGAVN (SEQ ID NO: 220), and a CDRH3 sequence including

RGKNCDYNWDFEHW (SEQ ID NO: 221); and a variable light chain including a CDRL1 sequence including QYGS, a CDRL2 sequence including SGS, and a CDRL3 sequence including QQYEF (SEQ ID NO: 222).

[0109] Exemplary anti-HIV antibodies also include ab18633 and 39/5.4A (available from ABCAM); and H81E (available from THERMOFISHER).

[0110] An example of an anti-Dengue virus antibody is antibody 55 described in U.S. 20170233460 and includes a variable heavy chain including a CDRH1 sequence including EVQLHQSGAELVKPGASVKLSCTVSGFNIK (SEQ ID NO: 223), a CDRH2 sequence including WVKQRPEQGLEWI (SEQ ID NO: 224), and a CDRH3 sequence including ATIKADTSSNTAYLQLISLTSEDTAVYYCAF (SEQ ID NO: 225); and a variable light chain including a CDRL1 sequence including DIQMTQSPASLSVSVGETVTITC (SEQ ID NO: 226), a CDRL2 sequence including WYQQKQGKSPQLLVY (SEQ ID NO: 227), and a CDRL3 sequence including GVPSRFSGSGSGTQYSLKINSLQSEDFGTYYC (SEQ ID NO: 228).

[0111] An additional example of an anti-Dengue virus antibody is DB2-3 described in U.S. Patent No. 8,637,035 and includes a variable heavy chain including a CDRH1 sequence including YTFTDYAIT (SEQ ID NO: 229), a CDRH2 sequence including GLISTYYGDSFYNQKFKG (SEQ ID NO: 230), and a CDRH3 sequence including TIRDGKAMDY (SEQ ID NO: 231); and a variable light chain including a CDRL1 sequence including RSSQSLVHSNGNTYLH (SEQ ID NO: 232), a CDRL2 sequence including KVSNRFS (SEQ ID NO: 233), and a CDRL3 sequence including SQSTHVPYT (SEQ ID NO: 234). Examples of anti-Dengue virus antibodies also include ab155042 and ab80914 (both available from ABCAM).

[0112] An example of an anti-pertussis antibody is described in U.S. Patent No. 9,512,204 and includes a variable heavy chain including QVQLQQPGSELVRPGASVKLSCKASGYKFTS YWMHWVKQRPGQGLEWIGNIFPGSGSTNYDEKFNSKATLTVDTSSNTAYMQLSSLTSEDSAV YYCTRWLSGAYFDYWGQGTTVTVSS (SEQ ID NO: 235) and a variable light chain including QIVLTQSPALMSASPGEKVTMTCSASSSVSFMYWYQQKPRSSPKPWIYLTSNLPSGVPARFSG SGSGTSYSLTISSMEAEDAATYYCQQWSSHPPTFGSGTKLEIK (SEQ ID NO: 236).

[0113] An example of an anti-hepatitis C antibody includes a variable heavy chain including a CDRH1 sequence including SYGMHW (SEQ ID NO: 237), a CDRH2 sequence including VIWLDGSNTYYADSVKGR (SEQ ID NO: 238), and a CDRH3 sequence including ARDIFTVARGVIIYFDY (SEQ ID NO: 239); and a variable light chain including a CDRL1 sequence including RASQSVSSYLA (SEQ ID NO: 240), a CDRL2 sequence including DASNRAT (SEQ ID NO: 241), and a CDRL3 sequence including QQRSNWVT (SEQ ID NO: 242). Examples of anti-hepatitis C antibodies also include MAB8694 (available from MILLIPORE) and C7-50

(available from ABCAM).

[0114] An example of an anti-influenza virus antibody is described U.S. Patent No. 9,469,685 and includes a variable heavy chain including a CDRH1 sequence including GMTSNSLA (SEQ ID NO: 243), a CDRH2 sequence including IIPVFETP (SEQ ID NO: 244), and a CDRH3 sequence including ATSAGGIVNYYLSFNI (SEQ ID NO: 245); and a variable light chain including a CDRL1 sequence including QTITTW (SEQ ID NO: 246), a CDRL2 sequence including KTS, and a CDRL3 sequence including QQYSTYSGT (SEQ ID NO: 247). An example of an anti-influenza virus antibody also includes C102 (available from THERMOFISHER).

[0115] An exemplary anti-MPV antibody includes MPE8.

[0116] Exemplary anti-CMV antibodies includes MCMV5322A, MCMV3068A, LJP538, and LJP539. RG7667 includes a mixture of MCMV5322A and MCMV3068A while CSJ148 includes a mixture of LJP538, and LJP539. See also, for example, Deng et al., Antimicrobial Agents and Chemotherapy 62(2) e01108-17 (Feb. 2018); and Dole et al., Antimicrobial Agents and Chemotherapy 60(5) 2881-2887 (May 2016).

[0117] An example of an anti-EBV antibody includes a variable heavy chain including an AMM01 CDRH1 sequence including YTFIHFGISW (SEQ ID NO: 248), an AMM01 CDRH2 sequence including IDTNNGNTNYAQSLQG (SEQ ID NO: 249), and an AMM01 CDRH3 sequence including RALEMGHRSGFPFDY (SEQ ID NO: 250); and a variable light chain including an AMM01 CDRL1 sequence including GGHNIGAKNVH (SEQ ID NO: 251), an AMM01 CDRL2 sequence including YDSDRPS (SEQ ID NO: 252), and an AMM01 CDRL3 sequence including CQVWDSGRGHPLYV (SEQ ID NO: 253).

[0118] An example of an anti-HSV antibody includes HSV8-N and MB66.

[0119] Exemplary anti-*Clostridium difficile* antibodies include actoxumab and bezlotoxumab. See also, for example, Wilcox et al., N Engl J Med 376(4) 305-317 (2017).

[0120] Commercially available anti-TNF antibodies include infliximab (Remicade® Centocor, Inc., Malvern, PA with biosimilars Inflectra® Pfizer, Kent, UK and Ixifi® Pfizer, New York, NY), adalimumab (Humira® Abbott Laboratories, Abbott Park, IL with biosimilars Amjevita® Amgen, Thousand Oaks, CA and Cyltezo® Boehringer Ingelheim Int'l, Ingelheim, DE), golimumab (Simponi® Johnson & Johnson Corp., New Brunswick, NJ), etanercept (Enbrel ® Immunex Corp, Thousand Oaks, CA with biosimilar Erelzi® Novartis AG, Basel, CH), and certolizumab-pegol (Cimzia® UCB Pharma, Brussels, BE).

[0121] In particular embodiments, the CDRs of infliximab include: heavy chain residues 26-37, 52-70, and 103-116 and light chain residues 24-39, 55-61, and 94-102. In particular embodiments, the heavy chain of infliximab begins with EVKLEESGGGLVQPGGSMK (SEQ ID NO: 254) and

the light chain begins with DILLTQSPAILSVSPGER (SEQ ID NO: 255).

[0122] In particular embodiments, infliximab includes a variable heavy chain including a CDRH1 sequence including IFSNHW (SEQ ID NO: 256), a CDRH2 sequence including RSKSINSATH (SEQ ID NO: 257), and a CDRH3 sequence including NYYGSTY (SEQ ID NO: 258); and a variable light chain including a CDRL1 sequence including FVGSSIH (SEQ ID NO: 259), a CDRL2 sequence including KYASESM (SEQ ID NO: 260), and a CDRL3 sequence including QSHSW (SEQ ID NO: 261).

[0123] In particular embodiments, adalimumab includes a variable heavy chain including a CDRH1 sequence including TFDDYA (SEQ ID NO: 262), a CDRH2 sequence including TWNSGHID (SEQ ID NO: 263), and a CDRH3 sequence including VSYLSTASSL (SEQ ID NO: 264); and a variable light chain including a CDRL1 sequence including GIRNYLA (SEQ ID NO: 265), a CDRL2 sequence including YAASTLQ (SEQ ID NO: 266), and a CDRL3 sequence including RYNRA (SEQ ID NO: 267).

[0124] In particular embodiments, certolizumab includes a variable heavy chain including a CDRH1 sequence including VFTDYG (SEQ ID NO: 268), a CDRH2 sequence including NTYIGEPI (SEQ ID NO: 269), and a CDRH3 sequence including GYRSYAM (SEQ ID NO: 270); and a variable light chain including a CDRL1 sequence including NVGTNVA (SEQ ID NO: 271), a CDRL2 sequence including YSASFLY (SEQ ID NO: 272), and a CDRL3 sequence including QYNIY (SEQ ID NO: 273).

[0125] Numerous additional antibody sequences are available and known to those of ordinary skill in the art that can be used within the teachings of the current disclosure. Sequence information for commercially available antibodies may be found in the Drug Bank database, the CAS Registry, and/or the RSCB Protein Data Bank. Moreover, nucleic acid sequences encoding portions of selected antibodies described herein can be easily derived by one of ordinary skill in the art.

[0126] (ii) Gene Editing Techniques and Cell Sorting. Gene editing systems allow control over the target sites of genetic therapies. Within the teachings of the current disclosure, any gene editing system capable of precise sequence targeting and modification can be used. These systems typically include a targeting element for precise targeting and a cutting element for cutting the targeted genetic site. Guide RNA is one example of a targeting element while various nucleases provide examples of cutting elements. Targeting elements and cutting elements can be separate molecules or linked, for example, by a nanoparticle. Alternatively, a targeting element and a cutting element can be linked together into one dual purpose molecule. When insertion of a therapeutic nucleic acid sequence is intended, the systems can also include homology-directed

repair templates (i.e., homology arms as described above) associated with the genetic construct. As detailed further below, however, different gene editing systems can adopt different components and configurations while maintaining the ability to precisely target, cut, and modify selected genomic sites.

[0127] Particular embodiments utilize zinc finger nucleases (ZFNs) as gene editing agents. ZFNs are a class of site-specific nucleases engineered to bind and cleave DNA at specific positions. ZFNs are used to introduce double strand breaks (DSBs) at a specific site in a DNA sequence which enables the ZFNs to target unique sequences within a genome in a variety of different cells. Moreover, subsequent to double-stranded breakage, homology-directed repair (HDR) or non-homologous end joining (NHEJ) takes place to repair the DSB, thus enabling genome editing.

[0128] ZFNs are synthesized by fusing a zinc finger DNA-binding domain to a DNA cleavage domain. The DNA-binding domain includes three to six zinc finger proteins which are transcription factors. The DNA cleavage domain includes the catalytic domain of, for example, FokI endonuclease. The FokI domain functions as a dimer requiring two constructs with unique DNA binding domains for sites on the target sequence. The FokI cleavage domain cleaves within a five or six base pair spacer sequence separating the two inverted half-sites.

[0129] For additional information regarding ZFNs, see Kim, et al. Proceedings of the National Academy of Sciences of the United States of America 93, 1156-1160 (1996); Wolfe, et al. Annual review of biophysics and biomolecular structure 29, 183-212 (2000); Bibikova, et al. Science 300, 764 (2003); Bibikova, et al. Genetics 161, 1169-1175 (2002); Miller, et al. The EMBO journal 4, 1609-1614 (1985); and Miller, et al. Nature biotechnology 25, 778-785 (2007).

[0130] Particular embodiments can use transcription activator like effector nucleases (TALENs) as gene editing agents. TALENs refer to fusion proteins including a transcription activator-like effector (TALE) DNA binding protein and a DNA cleavage domain. TALENs are used to edit genes and genomes by inducing DSBs in the DNA, which induce repair mechanisms in cells. Generally, two TALENs must bind and flank each side of the target DNA site for the DNA cleavage domain to dimerize and induce a DSB. The DSB is repaired in the cell by NHEJ or HDR if an exogenous double-stranded donor DNA fragment is present.

[0131] As indicated, TALENs have been engineered to bind a target sequence of, for example, an endogenous genome, and cut DNA at the location of the target sequence. The TALEs of TALENs are DNA binding proteins secreted by Xanthomonas bacteria. The DNA binding domain of TALEs include a highly conserved 33 or 34 amino acid repeat, with divergent residues at the 12th and 13th positions of each repeat. These two positions, referred to as the Repeat Variable Diresidue (RVD), show a strong correlation with specific nucleotide recognition. Accordingly,

targeting specificity can be improved by changing the amino acids in the RVD and incorporating nonconventional RVD amino acids.

[0132] Examples of DNA cleavage domains that can be used in TALEN fusions are wild-type and variant FokI endonucleases. For additional information regarding TALENs, see Boch, et al. Science 326, 1509-1512 (2009); Moscou, & Bogdanove, Science 326, 1501 (2009); Christian, et al. Genetics 186, 757-761 (2010); and Miller, et al. Nature biotechnology 29, 143-148 (2011).

[0133] Particular embodiments utilize MegaTALs as gene editing agents. MegaTALs have a single chain rare-cleaving nuclease structure in which a TALE is fused with the DNA cleavage domain of a meganuclease. Meganucleases, also known as homing endonucleases, are single peptide chains that have both DNA recognition and nuclease function in the same domain. In contrast to the TALEN, the megaTAL only requires the delivery of a single peptide chain for functional activity.

[0134] In particular embodiments, the endogenous B cell genome can be targeted using CRISPR gene editing systems. The CRISPR nuclease system is a prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages and provides a form of acquired immunity. CRISPRs are DNA loci containing short repetitions of base sequences. In the context of a prokaryotic immune system, each repetition is followed by short segments of spacer DNA belonging to foreign genetic elements that the prokaryote was exposed to. This CRISPR array of repeats interspersed with spacers can be transcribed into RNA. The RNA can be processed to a mature form and associate with a nuclease, such as cas (CRISPR-associated) nuclease. A CRISPR-Cas system including an RNA having a sequence that can hybridize to the foreign genetic elements and Cas nuclease can then recognize and cut these exogenous genetic elements in the genome.

[0135] A CRISPR-Cas system does not require the generation of customized proteins to target specific sequences, but rather a single Cas enzyme can be programmed by a short guide RNA molecule (crRNA) to recognize a specific DNA target. The CRISPR-Cas systems of bacterial and archaeal adaptive immunity show extreme diversity of protein composition and genomic loci architecture. The CRISPR-Cas system loci have more than 50 gene families and there are no strictly universal genes, indicating fast evolution and extreme diversity of loci architecture. So far, adopting a multi-pronged approach, there is comprehensive cas gene identification of 395 profiles for 93 Cas proteins. Classification includes signature gene profiles plus signatures of locus architecture. A new classification of CRISPR-Cas systems is proposed in which these systems are broadly divided into two classes, Class 1 with multi-subunit effector complexes and Class 2 with single-subunit effector modules exemplified by the Cas9 protein.

[0136] At least three different Cas9 nucleases have been developed for genome editing. The first is the wild type Cas9 which introduces double strand breaks (DSBs) at a specific DNA site, resulting in the activation of DSB repair machinery. DSBs can be repaired by non-homologous end joining (NHEJ) , homology-directed repair (HDR), or microhomology mediated repair (MMEJ). NHEJ can involve repair of a DSB with no homology (<5 bp) between the two ends joined during repair; HDR can involve repair of a DSB with a large region of homology between the ends joined during repair (100 or more nucleotides); and MMEJ can involve repair of a DSB with a small (5 to 50 bp) region of homology between the ends joined during repair. Another type of Cas9 includes a mutant Cas9, known as the Cas9D10A, with only nickase activity, which means that it only cleaves one DNA strand and does not activate NHEJ. Thus, the DNA repairs proceed via the HDR pathway only. The third is a nuclease-deficient Cas9 (dCas9) which does not have cleavage activity but is able to bind DNA. Therefore, dCas9 is able to target specific sequences of a genome without cleavage. By fusing dCas9 with various effector domains, dCas9 can be used either as a gene silencing or activation tool.

[0137] In addition to the Class 1 and Class 2 CRISPR-Cas systems, more recently a putative Class 2, Type V CRISPR-Cas class exemplified by Cpf1 has been identified Zetsche et al. (2015) Cell 163(3): 759-771. The Cpf1 nuclease particularly can provide added flexibility in target site selection by means of a short, three base pair recognition sequence (TTN), known as the protospacer-adjacent motif or PAM. Cpf1's cut site is at least 18bp away from the PAM sequence, thus the enzyme can repeatedly cut a specified locus after indel (insertion and deletion) formation, increasing the efficiency of HDR. Moreover, staggered DSBs with sticky ends permit orientation-specific donor template insertion.

[0138] Additional information regarding CRISPR-Cas systems and components thereof are described in, US8697359, US8771945, US8795965, US8865406, US8871445, US8889356, US8889418, US8895308, US8906616, US8932814, US8945839, US8993233 and US8999641 and applications related thereto; and WO2014/018423, WO2014/093595, WO2014/093622, WO2014/093635, WO2014/093655, WO2014/093661, WO2014/093694, WO2014/093701, WO2014/093709, WO2014/093712, WO2014/093718, WO2014/145599, WO2014/204723, WO2014/204724, WO2014/204725, WO2014/204726, WO2014/204727, WO2014/204728, WO2014/204729, WO2015/065964, WO2015/089351, WO2015/089354, WO2015/089364, WO2015/089419, WO2015/089427, WO2015/089462, WO2015/089465, WO2015/089473 and WO2015/089486, WO2016205711, WO2017/106657, WO2017/127807 and applications related thereto.

[0139] Particular embodiments combine tracrRNA and crRNA into a single synthetic single guide

RNA (sgRNA utilizing e.g., SEQ ID NOs: 87-89, or 290-366). In particular embodiments, an sgRNA can include a twenty nucleotide sequence that is analogous to the crRNA, and a tracrRNA sequence. For certain gene editing systems, the target sequence may be adjacent to a PAM (e.g., 5'- 20nt target – NGG-3'). In particular embodiments, a target sequence can include a PAM (SEQ ID NOs: 5-84). In particular embodiments, guide RNA (gRNA) includes a target site adjacent to the PAM targeted by the genome editing complex. The gRNA can include at least the 16, 17, 18, 19, 20, 21, or 22 nucleotides adjacent to the PAM.

[0140] In particular embodiments, a cutting element is directed to the targeted DNA location with the assistance of engineered gRNAs (FIG. 25A (Sternberg et al., Mol Cell. 2015;58(4):568-74)). Genetic constructs with homology arms flanking the cut genomic region are efficiently inserted into this location by the homology-directed DNA repair mechanism (see., e.g., FIG. 15B (Elliott et al., Mol Cell Biol. 1998;18(1):93-101). Using this approach expression of the endogenous antibody will be eliminated and genes encoding the selected antibody will be inserted into the targeted genetic location. This targeted insertion eliminates or significantly reduces the possibility of off-target effects resulting from random genetic insertion.

[0141] In particular embodiments, sgRNA targeting the mouse or human IgH of each endogenous antibody targets the region 100 bp downstream of the J region (FIG. 9). In the experimental examples, this region was targeted to express a version of the selected antibody palivizumab containing the C region from the endogenous genome (FIGs. 7 and 9). The crispr.mit.edu algorithm (Hsu et al., Nat Biotechnol. 2013;31(9):827-32) identified 22 targeting sequences for this region that are predicted to have little, if any, off-target binding. Individual targeting sequences can be inserted into the full-length sgRNA and mixed with a nuclease such as Cas9 immediately prior to incubation as described (Schumann et al., Proc Natl Acad Sci U S A. 2015;112(33):10437-42) and electroporated into B cells (Kim et al., J Immunol. 1979;122(2):549-54). Since cellular repair of DNA cut by Cas9 often results in loss of gene function (Symington & Gautier, Annu Rev Genet. 2011;45:247-71), efficient sgRNAs targeting antibody coding regions are expected to result in the appearance of some B cells lacking antibody, which can be easily assessed by flow cytometry. The activity of sgRNAs targeting intronic sequences can be assessed by sequencing, or through enzymatic assays such as the T7 endonuclease assay.

[0142] In particular embodiments, genome targeting and cutting elements can be administered through electroporation, nanoparticle-mediated delivery and/or viral vector delivery. Electroporation can be useful, for example, to deliver targeting elements and/or cutting elements because the membrane of the cell does not normally allow such foreign molecules into the cell. Electroporation sends an electric shock to the cells that temporarily allows such foreign molecules

to pass through the cell membrane.

[0143] In particular embodiments, genetic constructs for insertion can be administered through electroporation, nanoparticle-mediated delivery and/or viral vector delivery. Adeno-associated viral vectors include those derived from e.g., adenovirus 5 (Ad5), adenovirus 35 (Ad35), adenovirus 11 (Ad11), adenovirus 26 (Ad26), adenovirus 48 (Ad48) or adenovirus 50 (Ad50)), and adeno-associated virus (AAV; see, e.g., U.S. Pat. No. 5,604,090; Kay et al., Nat. Genet. 24:257 (2000); Nakai et al., Blood 91:4600 (1998)).

[0144] In particular embodiments, genome targeting and cutting elements can be administered through electroporation and genetic constructs for insertion can be administered through AAV-mediated delivery. In particular embodiments, genome targeting and cutting elements can be administered through nanoparticle-mediated delivery and genetic constructs for insertion can be administered through AAV-mediated delivery.

[0145] In particular embodiments, the genetic construct including a transgene can be mixed with a targeting element (e.g., sgRNA) and a cutting element (e.g., Cas9 or cpf1) immediately or shortly before electroporation. Selected antibody expression can be confirmed later (e.g., 3 days later) by measuring cell binding to fluorescently tagged target proteins by flow cytometry. Enrichment and analysis methodologies for detecting and analyzing epitope-specific B cells can be used. Pape et al., Science. 2011;331(6021):1203-7; Taylor et al., J Exp Med. 2012;209(3):597-606; Taylor et al., J Exp Med. 2012;209(11):2065-77; Haasken et al., J Immunol. 2013;191(3):1055-62; Taylor et al., J Immunol Methods. 2014;405:74-86; Nanton et al., Eur J Immunol. 2015;45(2):428-41; Hamilton et al., J Immunol. 2015;194(10):5022-34; Taylor et al., Science. 2015;347(6223):784-7). These methodologies allow detection of selected antibody-expressing B cells at frequencies as extraordinarily low as 0.00002% of the total B cell population (Taylor et al., Science. 2015;347(6223):784-7).

[0146] In particular embodiments, cells can be identified and/or sorted based on marker expression, before or after delivering the genetic construct. For example, it may be useful to isolate a particular type of B cells (e.g., memory B cells, antibody-secreting B cells, naïve B cells, B1 B cells, marginal zone B cells) from a sample prior to delivering the genetic construct. As another example, it may be useful to isolate B cells from other cells present in a blood sample. CD19 is an example of a protein expressed by B cells but few other cells of the body. By marking CD19 with a fluorescent molecule, B cells can be specifically identified. B220 is a useful marker to identify mouse B cells.

[0147] CD27 is an example of a protein expressed by memory but not naive human B cells. By marking CD27 with a fluorescent molecule, memory B cells can be identified.

[0148] CD21 is an example of a protein not expressed (or expressed to a low degree) by some memory human B cells with the capacity to quickly secrete antibody following infection. Low CD21 expression can be used to define B cells primed for plasma cell differentiation. By marking CD21 with a fluorescent molecule, these B cells can be specifically identified by for example, negative selection.

[0149] Human naïve B cells can be identified by the marker profile IgM+ IgD+ CD27-. Mouse naïve B cells can be identified by the marker profile CD38+ GL7- IgM+ IgD+. Human B1 B cells can be identified by the marker profile CD5+ CD43+. Mouse B1 B cells can be identified by the marker profile CD43+ B220LOW. Human marginal zone B cells can be identified by the marker profile CD21+++ IgM++ IgD- CD27+. Mouse marginal zone B cells can be identified by the marker profile CD21+++ IgM++ IgD-.

[0150] Particular embodiments may utilize the $CD19^{+}CD27^{+}CD21^{lo}$marker profile.

[0151] CD45 is a marker used for identifying and/or isolating cell types used in the experiments described herein. Different mouse strains express different versions of the protein called CD45, termed CD45.1 and CD45.2. In experiments disclosed herein, B cells from a mouse that expresses CD45.2 will be taken and transferred into a mouse that expresses CD45.1. By marking CD45.1 and CD45.2 with different fluorescent molecules, one can identify the cells that came from the donor animal because they express CD45.2 but not CD45.1.

[0152] Particular embodiments include sorting B cells after genetic modification based on expression of an exogenous light chain. For example, B cells that naturally express a kappa light chain can be modified to express a selected antibody that includes a lambda light chain. B cells that naturally express a lambda light chain can be modified to express a selected antibody that includes a kappa light chain. Sorting based on expression of an exogenous light chain will allow for isolation of only those B cells expressing the selected antibody. In particular embodiments, only those B cells that completely lack surface expression of their endogenous light chain are isolated for formulation and administration to a subject.

[0153] In particular embodiments, cells may be identified and/or isolated using flow cytometry. Flow cytometry is a sensitive and powerful analysis approach that uses lasers to individually analyze the fluorescent molecules marking millions of individual cells. By analyzing the combination of fluorescent molecules each cell is marked with, different B cell subtypes can be identified. Flow cytometry can be used to identify B cell subsets and analyze the expression of selected antibodies (e.g., palivizumab) within these cells.

[0154] In particular embodiments, methods of modifying B cells can include obtaining hematopoietic stem cells (HSC), and/or delivering the genetic constructs to HSC. HSC can refer

to a type of stem cell that naturally produces B cells as well as all other cells of the immune system. HSC can be obtained, for example, from cord blood.

[0155] Particular experimental results described herein utilized A20 cells to develop the genetic modification methodology prior to moving on to freshly-isolated B cells. A20 is an immortalized cell line made from a mouse B cell.

[0156] In particular embodiments, B cells may be obtained from a human subject and obtained B cells or a subset thereof may be modified ex vivo.

[0157] Formulations of Modified B Cells. Once modified, cells can be harvested from a culture medium, and washed and concentrated into a carrier in a therapeutically-effective amount. Exemplary carriers include saline, buffered saline, physiological saline, water, Hanks' solution, Ringer's solution, Nonnosol-R (Abbott Labs), PLASMA-LYTE A® (Baxter Laboratories, Inc., Morton Grove, IL), glycerol, ethanol, and combinations thereof.

[0158] In particular embodiments, carriers can be supplemented with human serum albumin (HSA) or other human serum components or fetal bovine serum. In particular embodiments, a carrier for infusion includes buffered saline with 5% hyaluronic acid sodium salt (HAS) or dextrose. Additional isotonic agents include polyhydric sugar alcohols including trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol, or mannitol.

[0159] Carriers can include buffering agents, such as citrate buffers, succinate buffers, tartrate buffers, fumarate buffers, gluconate buffers, oxalate buffers, lactate buffers, acetate buffers, phosphate buffers, histidine buffers, and/or trimethylamine salts.

[0160] Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which helps to prevent cell adherence to container walls. Typical stabilizers can include polyhydric sugar alcohols; amino acids, such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, and threonine; organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol, and cyclitols, such as inositol; PEG; amino acid polymers; sulfur-containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, alpha-monothioglycerol, and sodium thiosulfate; low molecular weight polypeptides (i.e., <10 residues); proteins such as HSA, bovine serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides such as xylose, mannose, fructose and glucose; disaccharides such as lactose, maltose and sucrose; trisaccharides such as raffinose, and polysaccharides such as dextran.

[0161] Where necessary or beneficial, formulations can include a local anesthetic such as lidocaine to ease pain at a site of injection.

[0162] Exemplary preservatives include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides, hexamethonium chloride, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol.

[0163] Formulations can include, for example, greater than 10^2 modified B cells, greater than 10^3 modified B cells, greater than 10^4 modified B cells, greater than 10^5 modified B cells, greater than 10^6 modified B cells, greater than 10^7 modified B cells, greater than 10^8 modified B cells, greater than 10^9 modified B cells, greater than 10^{10} modified B cells, or greater than 10^{11} modified B cells.

[0164] Methods of Use. Methods disclosed herein include treating subjects (e.g., humans, veterinary animals (dogs, cats, reptiles, birds) livestock (e.g., horses, cattle, goats, pigs, chickens) and research animals (e.g., monkeys, rats, mice, fish) with formulations disclosed herein. Treating subjects includes delivering therapeutically effective amounts. Therapeutically effective amounts include those that provide effective amounts, prophylactic treatments and/or therapeutic treatments.

[0165] An "effective amount" is the amount of a composition necessary to result in a desired physiological change in the subject. Effective amounts are often administered for research purposes. Effective amounts disclosed herein can cause a statistically-significant effect in an animal model or in vitro assay relevant to the assessment of a condition's development, progression, and/or resolution.

[0166] A "prophylactic treatment" includes a treatment administered to a subject who does not display signs or symptoms of a condition or displays only early signs or symptoms of a condition such that treatment is administered for the purpose of diminishing or decreasing the risk of developing the condition. Thus, a prophylactic treatment functions as a preventative treatment against a condition. In particular embodiments, prophylactic treatments reduce, delay, or prevent the worsening of a condition. Particular embodiments include administration of a formulation described herein as prophylactic protection in the absence of a currently effective vaccine. Particular embodiments include administration of a formulation described herein as prophylactic protection as a replacement for conventional vaccination strategies. Particular embodiments include administration of a formulation described herein as prophylactic protection as a supplement to conventional vaccination strategies.

[0167] A "therapeutic treatment" includes a treatment administered to a subject who displays symptoms or signs of a condition and is administered to the subject for the purpose of diminishing or eliminating those signs or symptoms of the condition. The therapeutic treatment can reduce, control, or eliminate the presence or activity of the condition and/or reduce control or eliminate

side effects of the condition.

[0168] In particular embodiments, the condition is an infection.

[0169] Function as an effective amount, prophylactic treatment or therapeutic treatment are not mutually exclusive, and in particular embodiments, administered dosages may accomplish more than one treatment type.

[0170] In particular embodiments, therapeutically effective amounts provide anti-pathogen effects. Anti-pathogen effects can include anti-infection effects. Anti-infection effects can include a decrease in the occurrence of infections, a decrease in the severity of infections, a decrease in the duration of infections, a decrease in the number of infected cells, a decrease in volume of infected tissue, an increase in life expectancy, induced sensitivity of infected cells to immune clearance, reduced infection-associated pain, and/or reduction or elimination of a symptom associated with the treated infection.

[0171] In particular embodiments, therapeutically effective amounts provide anti-inflammatory effects. Anti-inflammatory effects can include reduced inflammation-associated pain, heat, redness, swelling and/or loss of function.

[0172] In particular embodiments, therapeutically effective amounts provide anti-Crohn's disease effects or anti-ulcerative colitis effects. Anti-Crohn's disease effects or anti-ulcerative colitis effects can include reduced diarrhea, reduced rectal bleeding, reduced unexplained weight loss, reduced fever, reduced abdominal pain and cramping, reduced fatigue and feelings of low energy, and/or restored appetite.

[0173] In particular embodiments, therapeutically effective amounts provide anti-arthritis effects. Anti-arthritis effects can include reduced pain, stiffness, swelling, redness in the joints and/or a restored range of motion. Types of arthritis include rheumatoid arthritis (RA), ankylosing spondylitis, and psoriatic arthritis.

[0174] In particular embodiments, therapeutically effective amounts provide anti-plaque psoriasis effects. Anti-plaque psoriasis effects can include reduced red patches, scaling spots, itching, burning, soreness, nail bed abnormalities and/or swollen or stiff joints.

[0175] In particular embodiments, B cells may be obtained from a subject, a subset of the B cells may be modified ex vivo, and then the modified B cells may be formulated and administered to the subject. In particular embodiments, a first subset of the subject's B cells may be modified with a first genetic construct to produce a selected antibody against a first pathogen, and a second subset of the subject's B cells may be modified with a second genetic construct to produce a selected antibody against a second pathogen, thereby providing protective antibodies against two pathogens. As indicated, B cells against any number of pathogens can be formed and

administered to a subject. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-HIV antibody, an anti-Dengue virus antibody, an anti-*Bordatella pertussis* antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-MPV antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-*Clostridium difficile* bacterial toxin antibody, and/or an anti-TNF antibody. In particular embodiments, the selected antibodies can be one or more of an anti-RSV antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and/or an anti-MPV antibody. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and an anti-MPV antibody. In particular embodiments, the selected antibody is palivizumab.

[0176] In particular embodiments, B cells may be obtained from a bone marrow donor or a hematopoietic stem cell donor that has been immunologically matched to a recipient. In particular embodiments, a first subset of the donor's B cells may be modified with a first genetic construct to produce a selected antibody against a first pathogen, and a second subset of the donor's B cells may be modified with a second genetic construct to produce a selected antibody against a second pathogen, thereby providing protective antibodies against two pathogens. As indicated, B cells against any number of pathogens can be formed and administered to a subject. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-HIV antibody, an anti-Dengue virus antibody, an anti-*Bordatella pertussis* antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-MPV antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-*Clostridium difficile* bacterial toxin antibody, and/or an anti-TNF antibody. In particular embodiments, the selected antibodies can be one or more of an anti-RSV antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and/or an anti-MPV antibody. In particular embodiments, the selected antibodies can be an anti-RSV antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, and an anti-MPV antibody. In particular embodiments, the selected antibody is palivizumab. The genetically-modified B cells can be administered to the recipient to provide protection against infection (e.g., an anti-infection effect) until the transplanted cells repopulate the recipient's own immune system.

[0177] In particular embodiments, the recipient is receiving bone marrow from a donor or a hematopoietic stem cell transplant as a treatment for a hematological malignancy. Examples of hematological malignancies include acute lymphocytic leukemia, B-cell prolymphocytic leukemia, Burkitt lymphoma/leukemia, chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma, follicular lymphoma (grades I, II, III, or IV), Hodgkin's lymphoma, intravascular large B-cell

lymphoma, lymphoma, lymphoplasmocytic lymphoma, mantle cell lymphoma, marginal zone lymphoma (extra-nodal and nodal), mediastinal (thymic) large B-cell lymphoma, multiple myeloma, non-Hodgkin's lymphoma, POEMS syndrome / osteosclerotic myeloma, primary effusion lymphoma, splenic marginal zone lymphoma, small lymphocytic lymphoma, smoldering multiple myeloma (SMM), and Waldenstrom's macroglobulinemia.

[0178] In particular embodiments, the recipient is receiving genetically-modified hematopoietic stem cells that provide a gene the recipient is lacking. These recipients may have a primary or secondary immunodeficiency that can be treated with the provision of a therapeutic gene through hematopoietic stem cells. More than 80 primary immune deficiency diseases are recognized by the World Health Organization. These diseases are characterized by an intrinsic defect in the immune system in which, in some cases, the body is unable to produce any or enough antibodies against infection. In other cases, cellular defenses to fight infection fail to work properly. Typically, primary immune deficiencies are inherited disorders. X-linked severe combined immunodeficiency (SCID-X1) is another example of a primary immune deficiency. X-linked SCID results in both a cellular and humoral immune depletion caused by mutations in the common gamma chain gene (γC), which result in the absence of T and natural killer (NK) lymphocytes.

[0179] Secondary, or acquired, immune deficiencies are not the result of inherited genetic abnormalities, but rather occur in individuals in which the immune system is compromised by factors outside the immune system. Examples include trauma, viruses, chemotherapy, toxins, and pollution. Acquired immunodeficiency syndrome (AIDS) is an example of a secondary immune deficiency disorder caused by a virus, the human immunodeficiency virus (HIV), in which a depletion of T lymphocytes renders the body unable to fight infection.

[0180] In particular embodiments, B cells may be obtained from a subject, a subset of the B cells may be modified ex vivo, and then the modified B cells may be formulated and administered to the subject. In particular embodiments, a first subset of the subject's B cells may be modified with a first genetic construct to produce a selected antibody against an inflammatory molecule, such as an inflammatory cytokine, thereby providing antibodies that protect against inflammation. In particular embodiments, the selected antibodies can be anti-TNF antibodies and/or anti-IL-1 antibodies. In particular embodiments, the selected antibody is infliximab, adalimumab, and/or golimumab and/or an approved biosimilar thereof.

[0181] For administration, therapeutically effective amounts (also referred to herein as doses) can be initially estimated based on results from *in vitro* assays and/or animal model studies. Such information can be used to more accurately determine useful doses in subjects of interest. The actual dose amount administered to a particular subject can be determined by a physician,

veterinarian or researcher taking into account parameters such as physical and physiological factors including age, previous vaccinations (if any), target, body weight, severity of condition, type of condition, stage of condition, previous or concurrent therapeutic interventions, idiopathy of the subject and route of administration.

[0182] As indicated, in particular embodiments, modified B cells express a tag that allows, for example, tracking and/or elimination after administration to a subject

[0183] Exemplary doses can include greater than 10^2 modified B cells, greater than 10^3 modified B cells, greater than 10^4 modified B cells, greater than 10^5 modified B cells, greater than 10^6 modified B cells, greater than 10^7 modified B cells, greater than 10^8 modified B cells, greater than 10^9 modified B cells, greater than 10^{10} modified B cells, or greater than 10^{11} modified B cells.

[0184] In particular embodiments, the effects of selected antibodies can be measured using viral titers. Viral titer refers to the amount of virus that can be detected. High viral titers mean high levels of infection. An optimal protective response is observed with titers that fall to zero.

[0185] As will be understood by one of ordinary skill in the art, while particular embodiments have been described, additional embodiments may also be utilized within the scope of the disclosure. The following description provides description and enablement of representative additional embodiments.

Exemplary Embodiments.

1. A method of genetically engineering B cells to express a selected antibody including targeted insertion of a genetic construct including (i) a promoter and (ii) a transgene encoding a portion of a selected antibody at an intronic region that is constant in all B cells and that is (i) positioned relative to an enhancer element that interacts with the promoter to drive expression of the transgene; and (ii) in a configuration such that a portion of the B cells' endogenous antibody-encoding genome is not expressed.

2. A method of genetically engineering B cells to express a selected antibody including inserting into SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, a genetic construct including or encoding (i) a heavy chain promoter, (ii) a signal peptide, (iii) the full length light chain of the selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of the heavy chain of the selected antibody; and (vi) a splice junction, thereby genetically engineering the B cells to express the selected antibody.

3. A method of embodiment 1 or 2, wherein the B cells' endogenous variable heavy chain encoding genome is not excised during the genetic modification.

4. A method of any of embodiments 1-3, wherein the selected antibody is an anti-Respiratory Syncytial Virus (RSV) antibody, an anti-human immunodeficiency virus (HIV) antibody, an anti-

Dengue virus antibody, an anti-*Bordatella pertussis* antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-*Clostridium difficile* bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.

5. A method of any of embodiments 1-4, wherein the genetic construct includes SEQ ID NOs: 102-175, 278, 279, or 280-289.

6. A method of any of embodiments 2-5, wherein the flexible linker is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

7. A method of any of embodiments 2-6, wherein the flexible linker is selected from SEQ ID NOs: 180-184.

8. A method of any of embodiments 2-7, wherein the flexible linker is a Gly-Ser linker including 50-80 amino acids.

9. A method of any of embodiments 2-8, wherein the flexible linker is a Gly-Ser linker including 57 amino acids.

10. A method of any of embodiments 2-6, 8, or 9, wherein the flexible linker is SEQ ID NO: 122.

11. A method of any of embodiments 2-10, wherein the skipping element is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

12. A method of any of embodiments 2-11, wherein the skipping element is a self-cleaving peptide.

13. A method of embodiment 12, wherein the self-cleaving peptide is selected from SEQ ID NOs: 176-179.

14. A method of any of embodiments 2-13, wherein the skipping element is an internal ribosome entry site (IRES).

15. A method of any of embodiments 2-14, wherein the heavy chain promoter is selected from SEQ ID NOs: 111 and 128.

16. A method of any of embodiments 2-15, wherein the heavy chain promoter is IgVH1-69 or J558H10.

17. A method of any of embodiments 2-16, wherein the signal peptide is selected from SEQ ID NOs: 118, 134, and 185-194.

18. A method of any of embodiments 2-17, wherein the signal peptide is derived from human IgH heavy chain or human IgL light chain.

19. A method of any of embodiments 1-18, wherein the genetic construct includes homology

arms.

20. A method of embodiment 19, wherein the homology arms include SEQ ID NOs: 90-101, 110, 125, 127, 140, 142, 143, 153, 170, 171, 173, 174, 278, or 279.

21. A method of any of embodiments 1-20, wherein the genetic construct encodes a tag.

22. A method of embodiment 21, wherein the tag includes STREPTAG®, STREP® tag II, His tag, Flag tag, Xpress tag, Avi tag, calmodulin tag, polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, SBP tag, Softag 1, Softag 3, or V5 tag.

23. A method of embodiment 21 or 22, wherein the tag includes SEQ ID NOs: 122, or 195-204.

24. A method of any of embodiments 1-23, further including delivering a guide RNA (gRNA) sequence selected from one or more of SEQ ID NOs: 87-89, and 290-366, and a nuclease to the B cells.

25. A method of embodiment 24, wherein the delivering is through electroporation, a nanoparticle, or viral-mediated delivery.

26. A method of any of embodiments 1-25, wherein the genetic construct is part of an adeno-associated viral vector.

27. A method of any of embodiments 24-26, wherein the gRNA and nuclease are delivered through electroporation and the genetic construct is delivered as part of an adeno-associated viral vector.

28. A method of any of embodiments 24-27, wherein the nuclease is Cas9 or Cpf1.

29. A method of any of embodiments 24-28, wherein a target sequence targeted by one or more of the gRNA sequence is selected from one or more of SEQ ID NOs: 5-84 and the gRNA is selected from one or more of SEQ ID NOs: 87-89, and 290-366.

30. A method of any of embodiments 1-29, wherein the selected antibody is an anti-RSV antibody including palivizumab, AB1128, or ab20745.

31. A method of any of embodiments 1-30, wherein the selected antibody is: palivizumab including a heavy chain including SEQ ID NO: 138 and a light chain including SEQ ID NO: 136; palivizumab including a heavy chain including SEQ ID NO: 138 and a light chain including SEQ ID NO: 205; an anti-RSV antibody including a heavy chain including SEQ ID NO: 123 and a light chain including SEQ ID NO: 120; or an anti-RSV antibody including a heavy chain including SEQ ID NO: 123 and a light chain including SEQ ID NO: 206.

32. A method of any of embodiments 1-30, wherein the selected antibody is an anti-RSV antibody including a CDRH1 including SEQ ID NO: 207, a CDRH2 including SEQ ID NO: 208, a CDRH3 including SEQ ID NO: 209; a CDRL1 including SEQ ID NO: 210, a CDRL2 including SEQ ID NO: 211, and a CDRL3 including SEQ ID NO: 212.

33. A method of any of embodiments 1-29, wherein the selected antibody is an anti-HIV antibody including 10E8, VRC01, ab18633 or 39/5.4A.

34. A method of any of embodiments 1-29 or 33, wherein the selected antibody is an anti-HIV antibody including a heavy chain including SEQ ID NO: 150 and a light chain including SEQ ID NO: 149.

35. A method of any of embodiments 1-29 or 33, wherein the selected antibody is an anti-HIV antibody including a CDRH1 including SEQ ID NO: 213, a CDRH2 including SEQ ID NO: 214, a CDRH3 including SEQ ID NO: 215, a CDRL1 including SEQ ID NO: 216, a CDRL2 including SEQ ID NO: 217, and a CDRL3 including SEQ ID NO: 218 or a CDRH1 including SEQ ID NO: 219, a CDRH2 including SEQ ID NO: 220, a CDRH3 including SEQ ID NO: 221, a CDRL1 including QYGS, a CDRL2 including SGS, and a CDRL3 including SEQ ID NO: 222.

36. A method of any of embodiments 1-29, wherein the selected antibody is an anti-Dengue virus antibody including antibody 55, DB2-3, ab155042 or ab80914.

37. A method of any of embodiments 1-29 or 36, wherein the selected antibody is an anti-Dengue virus antibody including a CDRH1 including SEQ ID NO: 223, a CDRH2 including SEQ ID NO: 224, a CDRH3 including SEQ ID NO: 225; a CDRL1 including SEQ ID NO: 226, a CDRL2 including SEQ ID NO: 227, and a CDRKL3 including SEQ ID NO: 228 or a CDRH1 including SEQ ID NO: 229, a CDRH2 including SEQ ID NO: 230, a CDRH3 including SEQ ID NO: 231, a CDRL1 including SEQ ID NO: 232, a CDRL2 including SEQ ID NO: 233, and a CDRL3 including SEQ ID NO: 234.

38. A method of any of embodiments 1-29, wherein the selected antibody is an anti-pertussis antibody including a heavy chain including SEQ ID NO: 235 and a light chain including SEQ ID NO: 236.

39. A method of any of embodiments 1-29, wherein the selected antibody is an anti-hepatitis C antibody including MAB8694 or C7-50.

40. A method of any of embodiments 1-29 or 39, wherein the selected antibody is an anti-hepatitis C antibody including a CDRH1 including SEQ ID NO: 237, a CDRH2 including SEQ ID NO: 238, a CDRH3 including SEQ ID NO: 239, a CDRL1 including SEQ ID NO: 240, a CDRL2 including SEQ ID NO: 241, and a CDRL3 including SEQ ID NO: 242.

41. A method of any of embodiments 1-29, wherein the selected antibody is an anti-influenza virus antibody including C102.

42. A method of any of embodiments 1-29 or 41, wherein the selected antibody is an anti-influenza virus antibody including a heavy chain including SEQ ID NO: 159 and a light chain including SEQ ID NO: 158.

43. A method of any of embodiments 1-29 or 41, wherein the selected antibody is an anti-influenza virus antibody including a CDRH1 including SEQ ID NO: 243, a CDRH2 including SEQ ID NO: 244, a CDRH3 including SEQ ID NO: 245, a CDRL1 including SEQ ID NO: 246, a CDRL2 including KTS, and a CDRL3 including SEQ ID NO: 247.

44. A method of any of embodiments 1-29, wherein the selected antibody is an anti-MPV antibody including MPE8.

45. A method of any of embodiments 1-29, wherein the selected antibody is an anti-CMV antibody including MCMV5322A, MCMV3068A, LJP538, or LJP539.

46. A method of any of embodiments 1-29, wherein the selected antibody is an anti-EBV antibody including a heavy chain including SEQ ID NO: 168 and a light chain including SEQ ID NO: 166.

47. A method of any of embodiments 1-29, wherein the selected antibody is an anti-EBV antibody including a CDRH1 including SEQ ID NO: 248, a CDRH2 including SEQ ID NO: 249, a CDRH3 including SEQ ID NO: 250, a CDRL1 including SEQ ID NO: 251, a CDRL2 including SEQ ID NO: 252, and a CDRL3 including SEQ ID NO: 253.

48. A method of any of embodiments 1-29, wherein the selected antibody is an anti-HSV antibody including HSV8-N and MB66.

49. A method of any of embodiments 1-29, wherein the selected antibody is an anti-*Clostridium difficile* antibody including actoxumab or bezlotoxumab.

50. A method of any of embodiments 1-29, wherein the selected antibody is an anti-TNF antibody including infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.

51. A method of any of embodiments 1-29 or 50, wherein the selected antibody is an anti-TNF antibody including a heavy chain including SEQ ID NO: 254 and a light chain including SEQ ID NO: 255; a CDRH1 including SEQ ID NO: 256, a CDRH2 including SEQ ID NO: 257, and a CDRH3 including SEQ ID NO: 258; a CDRL1 including SEQ ID NO: 259, a CDRL2 including SEQ ID NO: 260, and a CDRL3 including SEQ ID NO: 261; a CDRH1 including SEQ ID NO: 262, a CDRH2 including SEQ ID NO: 263, and a CDRH3 including SEQ ID NO: 264; a CDRL1 including SEQ ID NO: 265, a CDRL2 including SEQ ID NO: 266, and a CDRL3 including SEQ ID NO: 267; a CDRH1 including SEQ ID NO: 268, a CDRH2 including SEQ ID NO: 269, and a CDRH3 including SEQ ID NO: 270; or a CDRL1 including SEQ ID NO: 271, a CDRL2 including SEQ ID NO: 272, and a CDRL3 including SEQ ID NO: 273.

52. A method of any of embodiments 1-51, wherein the genetic modification utilizes a sequence including any of SEQ ID NOs: 87, 88, 89, 90-175, 278-366.

53. A method of any of embodiments 1-52, wherein the B cell is an antibody-producing B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.

54. A B cell modified according to a method of any one of embodiments 1-53.

55. A B cell of embodiment 54, wherein the B cell is an antibody-secreting B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.

56. A method of providing an anti-infection effect in a subject in need thereof including administering a therapeutically effective amount of a B cell of embodiment 54 or 55 to the subject thereby providing an anti-infection effect.

57. A method of embodiment 56, wherein the providing obviates the need for a vaccination.

58. A method of embodiment 56 or 57, wherein the administering replaces a vaccination protocol.

59. A method of any of embodiments 56-58, wherein the subject is immune-suppressed.

60. A method of any of embodiments 56-59, wherein the subject is immune-suppressed as part of a treatment regimen including a bone marrow transplant, hematopoietic stem cell transplant, or administration of genetically modified hematopoietic stem cells.

61. A method of providing an anti-inflammatory effect in a subject in need thereof including administering a therapeutically effective amount of a B cell of embodiment 54 or 55 to the subject thereby providing an anti-inflammatory effect.

62. A genetic construct for modifying a B cell to express a selected antibody, the genetic construct including or encoding (i) a heavy chain promoter, (ii) a signal peptide, (iii) the full length light chain of the selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of the heavy chain of the selected antibody; and (vi) a splice junction.

63. A genetic construct of embodiment 62, including SEQ ID NOs: 102-175, or 280-289.

64. A genetic construct of embodiment 62 or 63, wherein the flexible linker is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

65. A genetic construct of any of embodiments 62-64, wherein the flexible linker is selected from SEQ ID NOs: 180-184.

66. A genetic construct of any of embodiments 62-65, wherein the flexible linker is a Gly-Ser linker including 50-80 amino acids.

67. A genetic construct of any of embodiments 62-66, wherein the flexible linker is a Gly-Ser linker includes 57 amino acids.

68. A genetic construct of any of embodiments 62-64, 66 or 67, wherein the flexible linker is SEQ ID NO: 122.

69. A genetic construct of any of embodiments 62-68, wherein the skipping element is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

70. A genetic construct of any of embodiments 62-69, wherein the skipping element is a self-cleaving peptide.

71. A genetic construct of embodiment 70, wherein the self-cleaving peptide is selected from SEQ ID NOs: 176-179.

72. A genetic construct of any of embodiments 62-69, wherein the skipping element is an internal ribosome entry site (IRES).

73. A genetic construct of any of embodiments 62-72, wherein the heavy chain promoter is selected from SEQ ID NOs: 111 and 128.

74. A genetic construct of any of embodiments 62-73, wherein the heavy chain promoter is IgVH1-69 or J558H10.

75. A genetic construct of any of embodiments 62-74, wherein the signal peptide is selected from SEQ ID NOs: 118, 134, and 185-194.

76. A genetic construct of any of embodiments 62-75, wherein the signal peptide is derived from human IgH heavy chain or human IgL light chain.

77. A genetic construct of any of embodiments 62-76, wherein the genetic construct includes homology arms.

78. A genetic construct of embodiment 77, wherein the homology arms include SEQ ID NOs: 90-101, 110, 125, 127, 140, 142, 143, 153, 170, 171, 173, 174, 278, or 279.

79. A genetic construct of any of embodiments 62-78, wherein the genetic construct encodes a tag.

80. A genetic construct of embodiment 79, wherein the tag includes STREPTAG®, STREP® tag II, His tag, Flag tag, Xpress tag, Avi tag, calmodulin tag, polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, SBP tag, Softag 1, Softag 3, or V5 tag.

81. A genetic construct of embodiment 79 or 80, wherein the tag includes SEQ ID NOs: 122, or 195-204.

82. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-RSV antibody including palivizumab, AB1128, or ab20745.

83. A genetic construct of any of embodiments 62-82, wherein the selected antibody is: palivizumab including a heavy chain including SEQ ID NO: 138 and a light chain including SEQ ID NO: 136; palivizumab including a heavy chain including SEQ ID NO: 138 and a light chain including SEQ ID NO: 205; an anti-RSV antibody including a heavy chain including SEQ ID NO: 123 and a light chain including SEQ ID NO: 120; or an anti-RSV antibody including a heavy chain including SEQ ID NO: 123 and a light chain including SEQ ID NO: 206.

84. A genetic construct of any of embodiments 62-82, wherein the selected antibody is an anti-

RSV antibody including a CDRH1 including SEQ ID NO: 207, a CDRH2 including SEQ ID NO: 208, a CDRH3 including SEQ ID NO: 209; a CDRL1 including SEQ ID NO: 210, a CDRL2 including SEQ ID NO: 211, and a CDRL3 including SEQ ID NO: 212.

85. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-HIV antibody including 10E8, VRC01, ab18633 or 39/5.4A.

86. A genetic construct of any of embodiments 62-81 or 85, wherein the selected antibody is an anti-HIV antibody including a heavy chain including SEQ ID NO: 150 and a light chain including SEQ ID NO: 149.

87. A genetic construct of any of embodiments 62-81 or 85, wherein the selected antibody is an anti-HIV antibody including a CDRH1 including SEQ ID NO: 213, a CDRH2 including SEQ ID NO: 214, a CDRH3 including SEQ ID NO: 215, a CDRL1 including SEQ ID NO: 216, a CDRL2 including SEQ ID NO: 217, and a CDRL3 including SEQ ID NO: 218 or a CDRH1 including SEQ ID NO: 219, a CDRH2 including SEQ ID NO: 220, a CDRH3 including SEQ ID NO: 221, a CDRL1 including QYGS, a CDRL2 including SGS, and a CDRL3 including SEQ ID NO: 222.

88. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-Dengue virus antibody including antibody 55, DB2-3, ab155042 or ab80914.

89. A genetic construct of any of embodiments 62-81 or 88, wherein the selected antibody is an anti-Dengue virus antibody including a CDRH1 including SEQ ID NO: 223, a CDRH2 including SEQ ID NO: 224, a CDRH3 including SEQ ID NO: 225; a CDRL1 including SEQ ID NO: 226, a CDRL2 including SEQ ID NO: 227, and a CDRKL3 including SEQ ID NO: 228 or a CDRH1 including SEQ ID NO: 229, a CDRH2 including SEQ ID NO: 230, a CDRH3 including SEQ ID NO: 231, a CDRL1 including SEQ ID NO: 232, a CDRL2 including SEQ ID NO: 233, and a CDRL3 including SEQ ID NO: 234.

90. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-pertussis antibody including a heavy chain including SEQ ID NO: 235 and a light chain including SEQ ID NO: 236.

91. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-hepatitis C antibody including MAB8694 or C7-50.

92. A genetic construct of any of embodiments 62-81 or 91, wherein the selected antibody is an anti-hepatitis C antibody including a CDRH1 including SEQ ID NO: 237, a CDRH2 including SEQ ID NO: 238, a CDRH3 including SEQ ID NO: 239, a CDRL1 including SEQ ID NO: 240, a CDRL2 including SEQ ID NO: 241, and a CDRL3 including SEQ ID NO: 242.

93. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-influenza virus antibody including C102.

94. A genetic construct of any of embodiments 62-81 or 93, wherein the selected antibody is an anti-influenza virus antibody including a heavy chain including SEQ ID NO: 159 and a light chain including SEQ ID NO: 158.

95. A genetic construct of any of embodiments 62-81 or 93, wherein the selected antibody is an anti-influenza virus antibody including a CDRH1 including SEQ ID NO: 243, a CDRH2 including SEQ ID NO: 244, a CDRH3 including SEQ ID NO: 245, a CDRL1 including SEQ ID NO: 246, a CDRL2 including KTS, and a CDRL3 including SEQ ID NO: 247.

96. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-MPV antibody including MPE8.

97. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-CMV antibody including MCMV5322A, MCMV3068A, LJP538, or LJP539.

98. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-EBV antibody including a heavy chain including SEQ ID NO: 168 and a light chain including SEQ ID NO: 166.

99. A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-EBV antibody including a CDRH1 including SEQ ID NO: 248, a CDRH2 including SEQ ID NO: 249, a CDRH3 including SEQ ID NO: 250, a CDRL1 including SEQ ID NO: 251, a CDRL2 including SEQ ID NO: 252, and a CDRL3 including SEQ ID NO: 253.

100.A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-HSV antibody including HSV8-N and MB66.

101.A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-*Clostridium difficile* antibody including actoxumab or bezlotoxumab.

102.A genetic construct of any of embodiments 62-81, wherein the selected antibody is an anti-TNF antibody including infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.

103.A genetic construct of any of embodiments 62-81 or 102, wherein the selected antibody is an anti-TNF antibody including a heavy chain including SEQ ID NO: 254 and a light chain including SEQ ID NO: 255; a CDRH1 including SEQ ID NO: 256, a CDRH2 including SEQ ID NO: 257, and a CDRH3 including SEQ ID NO: 258; a CDRL1 including SEQ ID NO: 259, a CDRL2 including SEQ ID NO: 260, and a CDRL3 including SEQ ID NO: 261; a CDRH1 including SEQ ID NO: 262, a CDRH2 including SEQ ID NO: 263, and a CDRH3 including SEQ ID NO: 264; a CDRL1 including SEQ ID NO: 265, a CDRL2 including SEQ ID NO: 266, and a CDRL3 including SEQ ID NO: 267; a CDRH1 including SEQ ID NO: 268, a CDRH2 including SEQ ID NO: 269, and a CDRH3 including SEQ ID NO: 270; or a CDRL1 including SEQ ID NO: 271, a CDRL2 including

SEQ ID NO: 272, and a CDRL3 including SEQ ID NO: 273.

104.A kit for genetically modifying a B cell including a genetic construct of any of embodiments 62-103 and a gRNA targeting SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

105.A kit of embodiment 104, wherein the gRNA is selected from one or more of SEQ ID NOs: 87, 88, 89, and 290-366.

106.A kit of embodiment 104 or 105, further including a nuclease.

107.A kit of embodiment 106, wherein the nuclease is Cas9 or Cpf1.

108.A kit of any of embodiments 104-107, further including a nanoparticle or adeno-associated viral vector.

109.A kit of any of embodiments 104-108, wherein the gRNA and nuclease are associated with a nanoparticle.

110.A kit of any of embodiments 104-109, wherein the genetic construct is part of an adeno-associated viral vector.

[0186] Example 1. Providing Life-Long Protection Against Respiratory Syncytial Virus Infection without a Vaccine. Respiratory syncytial virus (RSV) is a leading cause of severe respiratory illness in young children, particularly infants with chronic lung disease, congenital heart disease or born prematurely. Humoral immunity can mediate effective protection against RSV, demonstrated by the therapeutic effects of the recombinant antibody Synagis® (MedImmune, Inc.)/palivizumab. However, both natural infection and previous vaccine trials have failed to induce a fully protective immune response against RSV.

[0187] For RSV and other difficult to vaccinate against diseases, bypassing vaccination through engineering primary B cells to elicit expression of a desired therapeutic antibody is extremely attractive. The immunoglobin (Ig) loci are extremely large, diverse, and subject to extensive genomic recombination and editing. In addition, the transcription of immunoglobin genes to produce both membrane and secreted forms relies on the regulation of mRNA splicing and polyadenylation by regulatory DNA elements. This complexity has made viral transduction, the traditional approach for cellular engineering of lymphocytes, technically impractical for the production of therapeutic B cells.

[0188] Particular embodiments include a platform for rapid and selective reprogramming of primary B cell antibody specificity by single hit immunogenetic engineering. This platform takes advantage of the high activity of the microhomology mediated end joining DNA repair pathway in primary B cells to insert a fully synthetic hybrid double stranded/single stranded DNA template after creation of DNA breaks by Cas9/sgRNA riboproteins. Key to this approach is preservation of endogenous regulatory elements, which allows for native control of surface bound and secreted

antibody expression. Moreover, the strategy is not restricted to RSV. It can be possible to express antibodies protective against virtually any pathogen with just a single blood draw and subsequent cell infusion a few days later.

[0189] Materials and Methods. Design of sgRNA. sgRNAs targeting intronic sequences in the mouse and human IgH locus were designed using CrispRGold (crisprgold.mdc-berlin.de) and produced in a synthetic form incorporating 2'-O-methyl analogs and 3' phosphorothioate internucleotide linkages at the first three 5' and 3' terminal RNA residues (Synthego). Genome targeting sequences are as follows:

Mouse: UUAUACAGUAUCCGAUGCAU (SEQ ID NO: 87)

Human: GUCUCAGGAGCGGUGUCUGU (SEQ ID NO: 89)

[0190] Design and assembly of template sequences.

[0191] Human: Antibody construct included the IgVH1-69 heavy chain promoter region (SEQ ID NO: 111), the full-length codon optimized light chain of palivizumab (SEQ ID NO: 113 (nucleotide) and SEQ ID NO: 120 (amino acid)), a 57-amino acid glycine-serine linker containing 3 tandem copies of the Streptag II motif (SEQ ID NO: 116 (nucleotide) and SEQ ID NO: 122 (amino acid)), the codon optimized variable region of the palivizumab heavy chain (SEQ ID NO: 117 (nucleotide) and SEQ ID NO: 123 (amino acid)), and a splice junction with 60 bp of flanking sequence derived from the human IGHJ1 gene segment (SEQ ID NO: 124).

[0192] Mouse: mCherry template included the J5558H10 heavy chain promoter, the full codon optimized mCherry open reading frame, and the sv40 polyadenylation site. Antibody constructs included the J5558H10 heavy chain promoter (SEQ ID NO: 128, V.A Love et. al Molecular Immunology 2000), full length codon optimized antibody light chain (SEQ ID NO: 130 (nucleotide) and SEQ ID NO: 135 (amino acid)), a 57 amino acid glycine-serine linker containing two tandem copies of the Streptag II sequence (SEQ ID NO: 116 (nucleotide) and SEQ ID NO: 122 (amino acid)), codon optimized variable region of the heavy antibody chain (SEQ ID NO: 133 (nucleotide) and SEQ ID NO: 138 (amino acid)), and a splice junction with 60 bp of flanking sequence derived from the mouse IGHJ3 gene segment (SEQ ID NO: 139).

[0193] Annealing of stitching oligonucleotides (i.e., splicing oligonucleotides). Stitching oligonucleotides with 36 bp of complementarity to the pam-distal and pam-proximal non-target DNA strand and 50-100 bp of complementarity to the inserted template were produced synthetically, and pre-annealed to the DNA template before use (e.g., SEQ ID Nos: 96-101). The splicing oligonucleotides can provide "homology stitches" as shown in FIG. 15.

[0194] Adeno-associated virus (AAV) viral vector template delivery. AAV viral vectors contained the MND promoter, full codon optimized mCherry open reading frame, and the sv40

polyadenylation site flanked by either 2 mouse sgRNA recognition sites or 400 bp of homology. AAV virions were produced in 293T cells pseudotyped with AAV6 viral capsid, and purified by sucrose gradient centrifugation and stored at -80°C. For viral delivery of template DNA, concentrated AAV virus was added to a final volume of 10% of total culture volume 12hrs before electroporation.

[0195] Mouse B cell culture and electroporation. Base B cell medium included RPMI medium with 10% Fetal calf serum (Hyclone), 10mM HEPES (Gibco), 1 mM sodium pyruvate, (Gibco), 55 μM β-mercaptoethanol (Sigma), and 100 U/ml penicillin plus 100 μg/mL streptomycin (Gibco) except in antibiotic free steps as noted.

[0196] B cells were isolated from spleen and lymph nodes via negative selection with magnetic beads (Miltenyi) and cultured for 24 hours at 2×10^6 /ml in B cell medium supplemented with 100 ng/ml recombinant carrier free HA-tagged mouse CD40L (R&D systems), 100 ng/ml anti-HA antibody (clone 543851, R&D systems), and 4 ng/ml mouse IL-4 (R&D systems). Next, the B cells were electroporated using the Neon transfection system and 10-μl tip as follows. Cas9 protein (Invitrogen) and synthetic sgRNA (Synthego) were mixed at a ratio of 3 μg Cas9/900 ng sgRNA and incubated at room temperature for at least 10 minutes. B cells were washed with PBS and suspended in Neon Buffer T at a final density of 2.5×10^7 cells/ml with Cas9/sgRNA and the pre-assembled DNA template. Cells were electroporated (1675 V, 10 milliseconds, 3 pulses) and immediately dispensed into pre-warmed antibiotic free medium.

[0197] For cell expansion, B cells were co-cultured with irradiated (80 gy) 3T3- CD40L feeder cell in the presence of 20 ng/ml mouse IL-21.

[0198] Assessment of sgRNA activity by Tracking of Indels by Decomposition (TIDE). Total genomic DNA was isolated from mock and Cas9 treated cells at 3-5 days post electroporation. The 500-600 bp region flanking the cut site was amplified by PCR using the following oligos:

Mouse: Forward: GGCTCCACCAGACCTCTCTA (SEQ ID NO: 274)
Reverse: AACCTCAGTCACCGTCTCCT (SEQ ID NO: 275)
Human: Forward: ACAGTAAGCATGCCTCCTAAG (SEQ ID NO: 276)
Reverse: GCCACTCTAGGGCCTTTGTT (SEQ ID NO: 277)

[0199] Results. Results are shown in FIGs. 18A-18C, FIGs. 22A & 22B, FIGs. 23B-23E, and FIGs. 24A -24C. FIGs. 18A, 18B, and 18C demonstrate successful cutting of mouse B cell line A20, primary mouse B cells, and human B cell line RAMOS, respectively, at the target IgH loci after electroporation of cells with Cas9/sgRNA ribonuclear proteins. FIGs 22A and 22B depict insertion of a mCherry fluorescent protein reporter into the IgH locus of primary mouse B cells. FIGs. 23B and 23C demonstrate surface expression of an anti-RSV antibody after insertion of a partial

antibody cassette into A20 mouse B cell lines (FIG. 23B) and RAMOS human B cell lines (FIG. 23C). FIGs. 23D and 23E demonstrates secretion of an anti-RSV antibody after insertion of a partial antibody cassette into A20 mouse B cell lines (FIG. 23D) and RAMOS human B cell lines (FIG 23E). FIG 24A demonstrates surface expression of an anti-RSV antibody after insertion of a partial antibody cassette into primary mouse B cells, initially (left panels) and after enrichment and expansion in vitro (right panels). FIG. 24B demonstrates secretion of an anti-RSV antibody after insertion of a partial antibody cassette into primary mouse B cell lines. FIG. 24C demonstrates the *in vitro* proliferative potential of engineered B cells.

[0200] Example 2. The goal of this example was to produce genetically-modified B cells with a defined specificity which maintain native control of secreted and surface Ig expression through genome engineering of the IgH locus. The IgH locus in B cells is a difficult region to target for genome engineering, due to the highly variable sequences present in B cells. B cell development results in recombination of V, D, and J elements over more than 1 megabase of DNA to generate the VDJ variable regions fundamental to antibody diversity. Later in B cell ontogeny, class switch between different constant regions results in loss of DNA over a similar sequence range (Reviewed in Watson, et al., (2017). Trends Immunol 38(7): 459-470).

[0201] This sequence variability makes directly targeting antibody coding regions impractical. However, a small DNA region between the last J gene segment and the switch region involved in class switch is present in all B cells. This universal target contains the critical intronic Eμ enhancer, one of several strong enhancer elements which cooperate to drive high level expression of IgH genes, despite their weak promoters. Activity of these enhancers is regulated in part by the proximity of promoters relative to the Eμ enhancer, and insertion of a transgene between the recombined VDJ segments and the Eμ enhancer can completely block VDJ transcription (Delpy, et al., (2002). J Immunol 169(12): 6875-6882). For this reason, methods used in this example targeted the area upstream of the Eμ enhancer for insertion of a new antibody cassette (FIG. 26A). By targeting this region, inserted emAb genes can be driven by a native (but inserted) IgH promoter, maximizing the native control of immunoglobin expression.

[0202] To enable one-hit insertion and minimize off-target interactions, emAb constructs were expressed as a single chain fusion. This fusion consists of a full light chain sequence, linked to the variable region of the heavy chain with a 57 amino acid glycine-serine linker as has been described for single chain F(ab) fragments. (Koerber, et al., (2015). J Mol Biol 427(2): 576-586) (FIG. 26A). This linker contains 3 tandem repeats of the StreptagII motif, to facilitate the detection and enrichment of genetically-modified cells (Schmidt & Skerra (2007). Nat Protoc 2(6): 1528-1535). Physically linking the light and heavy chains minimizes the possibility of misspairing

between an inserted emAb and endogenous light chain. An optimized splice junction allows emAbs to splice to downstream endogenous IgH constant regions. This allows emAbs to be expressed as any of the heavy chain isotype classes.

[0203] The strategy was tested in the RAMOS human B cell line. This Burkitts-lymphoma derived B cell line natively expresses surface and secreted forms of IgM paired with a lambda light chain. In these experiments, expression of an engineered αRSV-emAb derived from Palivizumab was detected using monomeric RSV-F protein and streptactin, a modified streptavidin with high affinity for the Streptag II motifs in the linker. αRSV-emAb genetically-modified RAMOS cells expressed the engineered RSV-specific antibody, which could be detected on the surface of cells (FIG. 26B) and as a secreted form in the supernatant (FIG. 26C). To confirm that an emAb BCR assembles with secondary protein complexes crucial for BCR signaling, RAMOS cells were exposed to stimulation with multimerized RSV-F antigen. αRSV-emAb engineered but not control cells exhibited rapid and sustained calcium signaling in response to protein antigen (FIG. 26D). These data served to confirm the viability of the emAb engineering approach.

[0204] Next, human primary B cells were genetically-modified using a multistep process of expansion and differentiation (FIG. 27A). Electroporation of pre-complexed guide RNA and Cas9 lead to highly efficient cutting of genomic DNA, resulting in faulty repairs of this region in 70% of the target alleles analyzed across multiple independent donors (FIG. 27B). The sgRNA target site is strongly conserved in humans, with no reported single nucleotide polymorphisms reported at a frequency above 1% (FIG. 27C). Addition of an AAV delivered αRSV-emAb cassette efficiently reprogrammed human B cells to bind RSV-F protein (FIG. 27D). Notably, emAb B cells have been successfully produced from every human donor tested, with an average engineering rate of 24% (FIG. 27E). *In vitro* culture and differentiation during the production of emAb increased antibody secretion potential. Primed cells at day 2 expressed high levels of CD19, and low amounts of the plasma cell markers CD138, CD27, and CD138, in contrast to cells at day 18, which had lower levels of CD19, and increased CD38, CD27, and CD138 (FIG. 27F). Corresponding to these alterations in cell surface markers, differentiated emAb engineered B cells secrete substantial amount of targeted antibody (FIG. 27G). Taken together, these data demonstrate the ability to rapidly and efficiently engineer primary B cells to produce specific protective antibodies.

[0205] To demonstrate the flexible aspect of the platform, emAb cassettes derived from 3 additional broadly neutralizing anti-viral antibodies were tested, including the anti-HIV targeted VRC01, the EBV targeted AMM01, and the influenza HA-stem targeted MEDI8852. Primary B cells were efficiently reprogrammed with all 4 constructs, which included antibodies with both kappa (Palivizumab, VRC01, Medi8852) and lambda (AMM01) light chains (FIG. 28). These data

demonstrate the flexible and broadly applicable nature of the emAb platform.

[0206] Blocking production of the endogenous Ig heavy chain is important to maximize the production of emAb and minimize the potential for production of unknown endogenous antibodies from genetically-modified cells. The RAMOS B cell line endogenously expresses an IgH paired with a lambda light chain. Engineering these cells with an αRSV-emAb linked to a kappa light chain enables use of surface lambda light chain expression as an effective measure of IgH expression. In addition, RAMOS cells have undergone a c-myc translocation, disrupting one IgH allele, such that any emAb insertion will by necessity be in the productive allele (FIG. 29A). Input RAMOS cells express high levels of lambda light chain on the surface, whereas cells expressing the αRSV-emAb have almost completely lost lambda expression (FIG. 29B). These data indicate that emAb insertion on the productive allele can effectively block expression of an endogenous IgH. In almost all primary B cells, one IgH allele possesses a productive VDJ rearrangement, whereas the other allele did not undergo VDJ recombination, or was unproductively recombined. However, both these alleles possess potential sites for emAb insertion (FIG. 29C). To test the effects of emAb insertion, purified lambda light chain expressing primary B cells were genetically-modified with αRSV-emAb. Input cells continued to express the endogenous antibody paired with lambda light chain on the surface. In contrast, half of αRSV-emAb engineered B cells have lost lambda light chain expression (FIG. 29D). The differential patterns of expression seen in RAMOS and primary B cells suggest that emAb insertion can block endogenous IgH expression if inserted into the productive allele. Differential expression of surface light chain is an avenue for purification of cells which exclusively express an emAb construct. Alternatively, the potential for insertion at either allele offers the possibility of producing dual-antibody expressing emAb cells by either selection of initial pool of anti-viral memory B cells for engineering, or by insertion of a different cassette on each allele.

[0207] Having demonstrated the ability to engineer B cells, the protective capability of the cells in murine models of viral infection was next confirmed. Murine emAb B cells were produced using a process of priming, electroporation + emAb cassette delivery, and expansion similar to that used in human primary B cells (FIG. 30A). Electroporation in combination with pre-complexed guide RNA and Cas9, cutting was highly efficient, resulting in faulty repairs of this region in 80% of the DNA analyzed (FIG. 30B). Delivery of a murine αRSV-emAb cassette via AAV reproducibly modified mouse B cells, with 8-24% of murine B cells binding RSV-F (FIG. 30C, 30D). Insertion in 1-7% of cells was also achieved using double stranded DNA (dsDNA) containing short homology regions instead of AAV (FIG. 30C, 30D (see also Example 1), offering a potential for emAb engineering of B cells using purely synthetic components. High titers of secreted

engineered antibodies could also be detected in culture supernatants produced by both methodologies (FIG. 30E).

[0208] To test the potential for antiviral protection 1.5 x 10^7 genetically-modified mouse B cells were infused into wild-type Balbc/byJ mice, followed by a blood draw and RSV challenge (FIG. 31A). RSV-specific antibodies and genetically-modified B cells were present in the blood 6 days following the transfer of genetically-modified B cells (FIG. 31B, 31C). Importantly, mice receiving genetically-modified B cells were almost completely protected against RSV infection (FIG. 31D). This protection approached that afforded by the injection of Palivizumab 2 days before infection (FIG. 31D). Transfer of mixed human emAb cells targeting RSV and influenza to NOD-scid IL2Rgammanull (NSG) mice lead to serum titers of antibodies targeting both viruses (FIGs. 32A, 32B). These results show that genetically-modified B cells disclosed herein protect against viral infection.

[0209] Methods. Design of single-chain antibody templates sequences. *Human:* Antibody constructs included the IgVH1-69 heavy chain promoter region (SEQ ID NO: 111), full-length antibody light chain (e.g., SEQ ID NOs: 113, 145, 154, and 161 (nucleotide) and SEQ ID NOs: 119, 148, 157, and 165 (amino acid)), a 57 amino acid glycine-serine linker containing 3 tandem copies of the StreptagII motif (SEQ ID NO: 116 (nucleotide) and SEQ ID NO: 122 (amino acid)), variable region of the heavy chain (e.g., SEQ ID NOs: 117, 147, 156, and 164 (nucleotide) and SEQ ID NOs: 123, 150, 159, and 168 (amino acid)), and a splice junction with 60 base pairs of flanking sequence derived from matching IgHJ variable regions (e.g., SEQ ID NOs: 124 and 151).

[0210] *Mouse:* Antibody constructs included the J5558H10 heavy chain promoter (SEQ ID NO: 128, V.A Love et. al Molecular Immunology 2000), full length codon optimized antibody light chain (e.g., SEQ ID NO: 130 (nucleotide) and SEQ ID NO: 135 (amino acid)), a 57 amino acid glycine-serine linker containing three tandem copies of the streptag II sequence (SEQ ID NO: 116 (nucleotide) and SEQ ID NO: 122 (amino acid)), codon optimized variable region of the heavy antibody chain (e.g., SEQ ID NO: 133 (nucleotide) and SEQ ID NO: 138 (amino acid)), and a splice junction with 60 base pairs of flanking sequence derived from the mouse IGHJ3 gene segment (e.g., SEQ ID NO: 139).

[0211] Full sequences of exemplary antibody constructs are available in FIG. 25B-25I.

[0212] Production of recombinant AAV vectors. AAV vectors were generated by triple transfection of AAV vector, serotype 6 capsid, and adenoviral helper plasmids (pHelper) into HEK293T cells using PEI. At 24 hours post-transfection, media was changed to serum-free DMEM, and after 72 hours cells were collected, lysed by freeze-thaw, benzonase treated, purified over iodixanol gradient followed by concentration into PBS using an Amicon Ultra-15 column (EMD Millipore)

(Choi, et al., (2007). Curr Protoc Mol Biol Chapter 16: Unit 16 25). Titers of the viral stock were determined by qPCR of AAV genomes, and ranged from 5×10^{10} to 1×10^{12} per microliter (Aurnhammer, et al., (2012). Hum Gene Ther Methods 23(1): 18-28).

[0213] Production of murine dsDNA emAb templates.

[0214] αRSV-emAb templates were amplified and short homology regions added by modified DNA oligos as follows:

Forward primer: (contains a 5' phosphate, mouse genomic homology region in bold)

/5Phos/**ACCACCTCTGTGACAGCATTTATACAGTATCCGATG**GACAAGTGAGTGTCTCAGG TTAGGATTCT (SEQ ID NO: 278)

Reverse primer (contains phosphorothioate stabilized DNA bonds (*) mouse genomic homology region in bold)

T*A*A*AGAAAGTGCCCCACTCCACTCTTTGTCCCTATGCTTGACCACAATGAATACTCCCA CC (SEQ ID NO: 279)

dsDNA template was amplified by PCR, purified and concentrated using minElute PCR cleanup columns (Qiagen).

[0215] Cell lines. 3T3-msCD40L were obtained from Dr. Mark Connors at the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Cat#12535. 3T3 cells were cultured in DMEM medium with 10% fetal calf serum (Gibco), 100 U/ml penicillin plus 100 µg/mL streptomycin (Gibco), and G418 (350 µg/mL).

[0216] RAMOS cells were obtained from ATCC (CRL-1596™). RAMOS cells were cultured in RPMI medium with 10% Fetal calf serum (Gibco) and 100 U/ml penicillin plus 100 µg/mL streptomycin (Gibco).

[0217] Mouse B cell culture and electroporation. Base B cell medium included RPMI medium with 10% Fetal calf serum (Gemini Biosciences), 10mM HEPES (Gibco), 1 mM sodium pyruvate, (Gibco), 55 µM Beta-mercaptoethanol (Sigma), and 100 U/ml penicillin plus 100 µg/mL streptomycin (Gibco) except in antibiotic free steps as noted.

[0218] B cells were isolated from spleen and lymph nodes via negative selection with magnetic beads (Miltenyi) and cultured for 24 hours at 2×10^6 /ml in B cell medium supplemented with 100 ng/ml recombinant carrier free HA-tagged mouse CD40L (R&D systems), 100 ng/ml anti-HA antibody (clone 543851, R&D systems), and 4 ng/ml mouse IL-4 (R&D systems). Next, the B cells were electroporated using the Neon transfection system as follows. Cas9 protein (Invitrogen) and synthetic sgRNA (Synthego) were mixed at a ratio of 1 µg Cas9 to 300 ng sgRNA and incubated at room temperature for at least 10 minutes. B cells were washed with PBS and suspended in Neon Buffer T at a final density of of 2.5×10^7 cells/ml with 12 µg of Cas9 RNP/10^6 cells. For dsDNA

conditions, 7.5 µg dsDNA template/10^6 cells was also included in the electroporation. Cells were electroporated (1675 V, 10 milliseconds, 3 pulses) and immediately dispensed into pre-warmed antibiotic free medium. For AAV conditions, concentrated AAV in PBS was added up to 15% of final culture volume. After electroporation, B cells were expanded for an additional 48 hours with B cell medium supplemented with 100 ng/ml recombinant carrier free HA-tagged mouse CD40L (R&D systems), 100 ng/ml anti-HA antibody (clone 543851, R&D systems), 4 ng/ml mouse IL-4 (R&D systems), and 20 ng/ml mouse IL-21. (Biolegend). For secondary expansion, B cells were co-cultured with irradiated (80 gy) NIH 3T3- CD40L feeder cells in the presence of 20 ng/ml mouse IL-21. (Biolegend).

[0219] Human B cell culture and electroporation. Basal media for human B cell culture (hBCM) was in IMDM media, with 10% FBS (Gemini Biosciences), 100 U/ml penicillin and 100 µg/mL streptomycin (Gibco), except in antibiotic free steps as noted.

[0220] Human PBMCs were obtained through the Fred Hutchinson Cancer Research Center. Cells were thawed, and isolated using negative selection using the Militenyi B Cell Isolation Kit II (Human), according to the manufacturer's protocol. Isolated cells were resuspended at 0.5-1.0*10^6 cells/mL in hBCM supplemented with 100 ng/mL MEGACD40L (Enzo Life Sciences), 50 ng/mL recombinant IL-2 (Biolegend), 50 ng/mL IL-10 (Shenendoah Biotech), 10 ng/mL IL-15 (Shenandoah Biotech), 1 µg/mL CpG ODN 2006 (IDT).

[0221] After 48 hours of stimulation, cells were electroporated using the Neon Transfection System. Cas9 protein (Invitrogen) and H7 sgRNA (Synthego) were precomplexed at a 2:1 ratio in Buffer T for 20 minutes at room temperature. Cells were washed with PBS (Gibco) and resuspended in Buffer T at a final concentration of 2.5*10^7 cells/ml in Buffer T containing pre-complexed Cas9 RNP. The Cell-RNP mixture was loaded into a 10uL Neon Transfection Tip, and electroporated according to the manufacturer's protocol with the settings of 1750V, 20ms, and 1 pulse. Immediately after electroporation, cells were plated into stimulation media as described above, without antibiotics. After 30 minutes, AAV was added to a final concentration of 10-15% culture volume and mixed thoroughly. After 2-4 hours, cells were transferred to a larger culture dish to allow for further expansion.

[0222] Two days after electroporation, cells were stained with fluorochrome labeled antigen or streptactin and genetically-modified cells were selected. For secondary expansion, B cells were co-cultured with irradiated (80 gy) NIH 3T3-CD40L feeder cells in hBCM containing 5 µg/mL Human recombinant Insulin (Sigma), 50 µg/mL Transferrin (Sigma), 50 ng/mL recombinant IL-2 (Biolegend), 20 ng/mL IL-21 (Biolegend), and 10 ng/mL IL-15 (Shenandoah Biotech).

[0223] In order to promote differentiation to plasma cells, cells were transferred from expansion

conditions into fresh feeder-free culture conditions containing hBCM supplemented with 5 µg /mL Human recombinant Insulin (Sigma), 50 µg/mL Transferrin (Sigma), 500 U/mL Universal Type I IFN Protein (R&D Systems), 50 ng/mL IL-6 (Shenendoah Biotech), 10 ng/mL IL-15 (Shenendoah Biotech).

[0224] Assessment of sGRNA activity by TIDE. Total genomic DNA was isolated from mock and cas9/sgRNA treated cells at 3-5 days post electroporation. The 500-600 base pair region flanking the sgRNA target site was amplified by PCR using the following oligos:

Mouse:

Forward: GGCTCCACCAGACCTCTCTA (SEQ. ID NO: 274)

Reverse: AACCTCAGTCACCGTCTCCT (SEQ ID NO: 275)

Human:

Forward: ACAGTAAGCATGCCTCCTAAG (SEQ ID NO: 276)

Reverse: GCCACTCTAGGGCCTTTGTT (SEQ ID NO: 277)

[0225] Purified PCR product was Sanger sequenced, and the frequency of indels in Cas9/sgRNA electroporated cells relative to mock electroporated cells was determined using the ICE algorithm (Hsiau, et al., (2018). "Inference of CRISPR Edits from Sanger Trace Data." bioRxiv).

[0226] Protein antigens. Pre-fusion RSV-F protein, EBV gh/gl complex, and modified HIV env antigen (426c TM4 d1-3) were produced as described (McLellan, et al., (2013). Science 342(6158): 592-598; McGuire, et al., (2016). Nat Commun 7: 10618; Snijder, et al., (2018). Immunity 48(4): 799-811 e799). Stabilized influenza HA-stem was produced from VRC clone 3925, derived from strain H1 1999 NC as described (Yassine, et al., (2015). Nat Med 21(9): 1065-1070). Monomeric prefusion RSV-F protein was labeled with Alexa-488 (Thermo Fisher). All other proteins were conjugated to biotin using a molar ratio of biotin:protein between 0.8 to 2, followed by tetramerization with streptavidin-PE or -APC (prozyme)

[0227] Flow Cytometry. Flow cytometric analysis was done on an FACSymphony machine (BD bioscience), cells were sorted on Aria II (BD bioscience), and data analyzed using FlowJo software (Tree Star).

[0228] EmAb therapeutic studies in mice. Animal studies were approved and conducted in accordance with the Fred Hutchinson Cancer Center Institutional Animal Care and Use Committee.

[0229] For RSV challenge, EmAb or control B cells were administered as a single intraperitoneal (IP) dose of 1.5×10^7 cells. For passive transfer of palivizumab, mice received a single dose of 15 mg/kg i.p. GFP-expressing RSV (here-in referred to as RSV for simplicity) was generously provided (Munir, et al., (2008). J Virol 82(17): 8780-8796). Age matched BALB/cByJ mice

(Jackson Labs) were inoculated intranasally with 10^6 *pfu* of sucrose purified RSV in 40 µL PBS. Lungs were harvested on day 5 post-infection and the titer was determined as previously described by plaque assay (Murphy, et al., (1990). Vaccine 8(5): 497-502). In brief, lungs were homogenized in 2 mls media in a GentleMACS dissociator, clarified by centrifugation at 400×g for 10 minutes, then flash frozen and stored at -80° C. The supernatant was diluted 1:10 and 1:20 in DMEM media in duplicate. 100 µL of each dilution was added to confluent Vero cells in 24 well plates for 2 hours at 37°C. An overlay of 0.8% methylcellulose was then added, and plates incubated for 5 days prior to imaging on a Typhoon imager with filter settings for GFP. The titer in pfu/lung was calculated by counting the number of plaques in the highest positive dilution and correcting for the dilution factor.

[0230] For engraftment of human cells, human emAb B cells were administered as a single IP dose of 5×10^6 cells/ emAb specificity (1×10^7 total) to NOD-*scid* IL2Rgamma^{null} (NSG) mice (produced by FHCRC breeding facility). 7 days post transfer, blood was drawn, and human emAb titers to RSV-F and HA-stem in serum determined by ELISA.

[0231] Statistical Analysis. Statistical analysis were performed using GraphPad Prism 7. Pairwise comparisons were performed using unpaired t-test with Welch's correction.

[0232] Nucleic acid sequences described herein are shown using standard letter abbreviations for nucleotide bases, as defined in 37 C.F.R. §1.822. In some instances, only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included in embodiments where it would be appropriate. By way of example, sequences complementary to target sites including SEQ ID NOs: 5-84 provide gRNA targeting sequences to target these sites.

[0233] Any nucleic acid that encodes a selected antibody construct as described herein may be utilized. Variants of nucleic acid sequences disclosed herein include various sequence polymorphisms, mutations, and alterations wherein the differences in the sequence do not substantially affect the function of the encoded protein. The term nucleic acid or "gene" may include not only coding sequences but also regulatory regions such as promoters, enhancers, and termination regions. The term further can include all introns and other DNA sequences spliced from the mRNA transcript, along with variants resulting from alternative splice sites. Encoding nucleic acid can be DNA or RNA that directs the expression of the one or more selected antibody constructs. These nucleic acid sequences may be a DNA strand sequence that is transcribed into RNA or an RNA sequence that is translated into protein. The nucleic acid sequences include both the full-length nucleic acid sequences as well as non-full-length sequences derived from the full-length protein. The sequences can also include degenerate codons of the native sequence or sequences that may be introduced to provide codon preference in a specific cell type. Nucleic

acid sequences encoding selected antibody constructs can be readily prepared from the relevant amino acid sequence of a selected antibody construct.

[0234] "Variants" of protein sequences include those having one or more amino acid additions, deletions, stop positions, or substitutions, as compared to a protein sequence disclosed elsewhere herein.

[0235] An amino acid substitution can be a conservative or a non-conservative substitution. Variants of protein sequence disclosed herein can include those having one or more conservative amino acid substitutions. A "conservative substitution" or "conservative amino acid substitution" involves a substitution found in one of the following conservative substitutions groups: Group 1: A, G, S, T; Group 2: D, E; Group 3: N, Q; Group 4: R, K, H; Group 5: I, L, M, V; and Group 6: F, Y, W.

[0236] Additionally, amino acids can be grouped into conservative substitution groups by similar function, chemical structure, or composition (e.g., acidic, basic, aliphatic, aromatic, or sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, G, A, V, L, and I. Other groups including amino acids that are considered conservative substitutions for one another include: sulfur-containing: M and C; acidic: D, E, N, and Q; small aliphatic, nonpolar or slightly polar residues: A, S, T, P, and G; polar, negatively charged residues and their amides: D, N, E, and Q; polar, positively charged residues: H, R, and K; large aliphatic, nonpolar residues: M, L, I, V, and C; and large aromatic residues: F, Y, and W.

[0237] Non-conservative substitutions include those that significantly affect: the structure of the peptide backbone in the area of the alteration (e.g., the alpha-helical or beta-sheet structure); the charge or hydrophobicity of the molecule at the target site; or the bulk of the side chain. Non-conservative substitutions which in general are expected to produce the greatest changes in the protein's properties are those in which (i) a hydrophilic residue (e.g. S or T) can be substituted for (or by) a hydrophobic residue (e.g. L, I, F, V, or A); (ii) a C or P can be substituted for (or by) any other residue; (iii) a residue having an electropositive side chain (e.g. K, R, or H) can be substituted for (or by) an electronegative residue (e.g. Q or D); or (iv) a residue having a bulky side chain (e.g. F), can be substituted for (or by) one not having a bulky side chain, (e.g. G). Additional information is found in Creighton (1984) Proteins, W.H. Freeman and Company.

[0238] Variants of nucleic acid and protein sequences disclosed herein also include sequences with at least 70% sequence identity, at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, or at least 99% sequence identity to a reference sequence disclosed herein.

[0239] "Percent(%)sequence identity" with respect to the sequences identified herein is defined as the percentage of nucleic acid or amino acid residues in a candidate sequence that are identical with the nucleic acid or amino acid residues in a reference sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid or amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For example, % sequence identity values generated using the WU-BLAST-2 computer program (Altschul et al., *Methods in Enzymology*, 266:460-480 (1996)) uses several search parameters, most of which are set to the default values. Those that are not set to default values (i.e., the adjustable parameters) are set with the following values: overlap span = 1, overlap fraction = 0.125, word threshold (T) = 11 and scoring matrix BLOSUM62.

[0240] Variants will typically exhibit the same qualitative biological activity and elicit a substantially similar biological response as a reference nucleic acid or peptide sequence, although variants can be selected to modify the characteristics of a reference nucleic acid or peptide as needed. Screening of variants can be performed using experimental protocols described herein.

[0241] As will be understood by one of ordinary skill in the art, each embodiment disclosed herein can comprise, consist essentially of or consist of its particular stated element, step, ingredient or component. Thus, the terms "include" or "including" should be interpreted to recite: "comprise, consist of, or consist essentially of." The transition term "comprise" or "comprises" means includes, but is not limited to, and allows for the inclusion of unspecified elements, steps, ingredients, or components, even in major amounts. The transitional phrase "consisting of" excludes any element, step, ingredient or component not specified. The transition phrase "consisting essentially of" limits the scope of the embodiment to the specified elements, steps, ingredients or components and to those that do not materially affect the embodiment. A material effect would cause a statistically significant reduction in B cell expression of a selected antibody.

[0242] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be

obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. When further clarity is required, the term "about" has the meaning reasonably ascribed to it by a person skilled in the art when used in conjunction with a stated numerical value or range, i.e. denoting somewhat more or somewhat less than the stated value or range, to within a range of ±20% of the stated value; ±19% of the stated value; ±18% of the stated value; ±17% of the stated value; ±16% of the stated value; ±15% of the stated value; ±14% of the stated value; ±13% of the stated value; ±12% of the stated value; ±11% of the stated value; ±10% of the stated value; ±9% of the stated value; ±8% of the stated value; ±7% of the stated value; ±6% of the stated value; ±5% of the stated value; ±4% of the stated value; ±3% of the stated value; ±2% of the stated value; or ±1% of the stated value.

[0243] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0244] The terms "a," "an," "the" and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0245] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the

specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0246] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0247] Furthermore, numerous references have been made to patents, printed publications, journal articles and other written text throughout this specification (referenced materials herein). Each of the referenced materials are individually incorporated herein by reference in their entirety for their referenced teaching.

[0248] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

[0249] The particulars shown herein are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of various embodiments of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for the fundamental understanding of the invention, the description taken with the drawings and/or examples making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

[0250] Definitions and explanations used in the present disclosure are meant and intended to be controlling in any future construction unless clearly and unambiguously modified in the following examples or when application of the meaning renders any construction meaningless or essentially meaningless. In cases where the construction of the term would render it meaningless or essentially meaningless, the definition should be taken from Webster's Dictionary, 3rd Edition or a dictionary known to those of ordinary skill in the art, such as the Oxford Dictionary of

Biochemistry and Molecular Biology (Ed. Anthony Smith, Oxford University Press, Oxford, 2004).

CLAIMS

What is claimed is:

1. A method of genetically modifying B cells to express Palivizumab comprising inserting into SEQ ID NO: 1 a genetic construct comprising a heavy chain promoter and nucleic acid sequences encoding the entire light chain of Palivizumab, a Gly-Ser linker, and the variable region of the heavy chain of Palivizumab thereby genetically engineering the B cells to express Palivizumab.

2. A method of genetically engineering B cells to express a selected antibody comprising inserting into SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4, a genetic construct comprising or encoding (i) a heavy chain promoter, (ii) a signal peptide, (iii) the full length light chain of the selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of the heavy chain of the selected antibody; and (vi) a splice junction, thereby genetically engineering the B cells to express the selected antibody.

3. A method of claim 2, wherein the B cells' endogenous variable heavy chain encoding genome is not excised during the genetic modification.

4. A method of claim 2, wherein the selected antibody is an anti-Respiratory Syncytial Virus (RSV) antibody, an anti-human immunodeficiency virus (HIV) antibody, an anti-Dengue virus antibody, an anti-*Bordatella pertussis* antibody, an anti-hepatitis C antibody, an anti-influenza virus antibody, an anti-parainfluenza virus antibody, an anti-metapneumovirus (MPV) antibody, an anti-cytomegalovirus antibody, an anti-Epstein Barr virus antibody; an anti-herpes simplex virus antibody, an anti-*Clostridium difficile* bacterial toxin antibody, or an anti-tumor necrosis factor (TNF) antibody.

5. A method of claim 2, wherein the genetic construct comprises SEQ ID NOs: 102-175, 278, 279, or 280-289.

6. A method of claim 2, wherein the flexible linker is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

7. A method of claim 2, wherein the flexible linker is selected from SEQ ID NOs: 180-184.

8. A method of claim 2, wherein the flexible linker is a Gly-Ser linker comprising 50-80 amino acids.

9. A method of claim 2, wherein the flexible linker is a Gly-Ser linker comprising 57 amino acids.

10. A method of claim 2, wherein the flexible linker is SEQ ID NO: 122.

11. A method of claim 2, wherein the skipping element is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

12. A method of claim 2, wherein the skipping element is a self-cleaving peptide.

13. A method of claim 12, wherein the self-cleaving peptide is selected from SEQ ID NOs: 176-179.

14. A method of claim 2, wherein the skipping element is an internal ribosome entry site (IRES).

15. A method of claim 2, wherein the heavy chain promoter is selected from SEQ ID NOs: 111 and 128.

16. A method of claim 2, wherein the heavy chain promoter is IgVH1-69 or J558H10.

17. A method of claim 2, wherein the signal peptide is selected from SEQ ID NOs: 118, 134, and 185-194.

18. A method of claim 2, wherein the signal peptide is derived from human IgH heavy chain or human IgL light chain.

19. A method of claim 2, wherein the genetic construct comprises homology arms.

20. A method of claim 19, wherein the homology arms comprise SEQ ID NOs: 90-101, 110, 125, 127, 140, 142, 143, 153, 170, 171, 173, 174, 278, or 279.

21. A method of claim 2, wherein the genetic construct encodes a tag.

22. A method of claim 21, wherein the tag comprises STREPTAG®, STREP® tag II, His tag, Flag tag, Xpress tag, Avi tag, calmodulin tag, polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, SBP tag, Softag 1, Softag 3, or V5 tag.

23. A method of claim 21, wherein the tag comprises SEQ ID NOs: 122, or 195-204.

24. A method of claim 2, further comprising delivering a guide RNA (gRNA) sequence selected from one or more of SEQ ID NOs: 87-89, and 290-366, and a nuclease to the B cells.

25. A method of claim 24, wherein the delivering is through electroporation, a nanoparticle, or viral-mediated delivery.

26. A method of claim 2, wherein the genetic construct is part of an adeno-associated viral vector.

27. A method of claim 24, wherein the gRNA and nuclease are delivered through electroporation and the genetic construct is delivered as part of an adeno-associated viral vector.

28. A method of claim 24, wherein the nuclease is Cas9 or Cpf1.

29. A method of claim 24, wherein a target sequence targeted by one or more of the gRNA sequence is selected from one or more of SEQ ID NOs: 5-84 and the gRNA is selected from one or more of SEQ ID NOs: 87-89, and 290-366.

30. A method of claim 2, wherein the selected antibody is an anti-RSV antibody comprising palivizumab, AB1128, or ab20745.

31. A method of claim 2, wherein the selected antibody is: palivizumab comprising a heavy chain comprising SEQ ID NO: 138 and a light chain comprising SEQ ID NO: 136; palivizumab comprising a heavy chain comprising SEQ ID NO: 138 and a light chain comprising SEQ ID NO:

205; an anti-RSV antibody comprising a heavy chain comprising SEQ ID NO: 123 and a light chain comprising SEQ ID NO: 120; or an anti-RSV antibody comprising a heavy chain comprising SEQ ID NO: 123 and a light chain comprising SEQ ID NO: 206.

32. A method of claim 2, wherein the selected antibody is an anti-RSV antibody comprising a CDRH1 comprising SEQ ID NO: 207, a CDRH2 comprising SEQ ID NO: 208, a CDRH3 comprising SEQ ID NO: 209; a CDRL1 comprising SEQ ID NO: 210, a CDRL2 comprising SEQ ID NO: 211, and a CDRL3 comprising SEQ ID NO: 212.

33. A method of claim 2, wherein the selected antibody is an anti-HIV antibody comprising 10E8, VRC01, ab18633 or 39/5.4A.

34. A method of claim 2, wherein the selected antibody is an anti-HIV antibody comprising a heavy chain comprising SEQ ID NO: 150 and a light chain comprising SEQ ID NO: 149.

35. A method of claim 2, wherein the selected antibody is an anti-HIV antibody comprising a CDRH1 comprising SEQ ID NO: 213, a CDRH2 comprising SEQ ID NO: 214, a CDRH3 comprising SEQ ID NO: 215, a CDRL1 comprising SEQ ID NO: 216, a CDRL2 comprising SEQ ID NO: 217, and a CDRL3 comprising SEQ ID NO: 218 or a CDRH1 comprising SEQ ID NO: 219, a CDRH2 comprising SEQ ID NO: 220, a CDRH3 comprising SEQ ID NO: 221, a CDRL1 comprising QYGS, a CDRL2 comprising SGS, and a CDRL3 comprising SEQ ID NO: 222.

36. A method of claim 2, wherein the selected antibody is an anti-Dengue virus antibody comprising antibody 55, DB2-3, ab155042 or ab80914.

37. A method of claim 2, wherein the selected antibody is an anti-Dengue virus antibody comprising a CDRH1 comprising SEQ ID NO: 223, a CDRH2 comprising SEQ ID NO: 224, a CDRH3 comprising SEQ ID NO: 225; a CDRL1 comprising SEQ ID NO: 226, a CDRL2 comprising SEQ ID NO: 227, and a CDRKL3 comprising SEQ ID NO: 228 or a CDRH1 comprising SEQ ID NO: 229, a CDRH2 comprising SEQ ID NO: 230, a CDRH3 comprising SEQ ID NO: 231, a CDRL1 comprising SEQ ID NO: 232, a CDRL2 comprising SEQ ID NO: 233, and a CDRL3 comprising SEQ ID NO: 234.

38. A method of claim 2, wherein the selected antibody is an anti-pertussis antibody comprising a heavy chain comprising SEQ ID NO: 235 and a light chain comprising SEQ ID NO: 236.

39. A method of claim 2, wherein the selected antibody is an anti-hepatitis C antibody comprising MAB8694 or C7-50.

40. A method of claim 2, wherein the selected antibody is an anti-hepatitis C antibody comprising a CDRH1 comprising SEQ ID NO: 237, a CDRH2 comprising SEQ ID NO: 238, a CDRH3 comprising SEQ ID NO: 239, a CDRL1 comprising SEQ ID NO: 240, a CDRL2 comprising SEQ ID NO: 241, and a CDRL3 comprising SEQ ID NO: 242.

41. A method of claim 2, wherein the selected antibody is an anti-influenza virus antibody comprising C102.

42. A method of claim 2, wherein the selected antibody is an anti-influenza virus antibody comprising a heavy chain comprising SEQ ID NO: 159 and a light chain comprising SEQ ID NO: 158.

43. A method of claim 2, wherein the selected antibody is an anti-influenza virus antibody comprising a CDRH1 comprising SEQ ID NO: 243, a CDRH2 comprising SEQ ID NO: 244, a CDRH3 comprising SEQ ID NO: 245, a CDRL1 comprising SEQ ID NO: 246, a CDRL2 comprising KTS, and a CDRL3 comprising SEQ ID NO: 247.

44. A method of claim 2, wherein the selected antibody is an anti-MPV antibody comprising MPE8.

45. A method of claim 2, wherein the selected antibody is an anti-CMV antibody comprising MCMV5322A, MCMV3068A, LJP538, or LJP539.

46. A method of claim 2, wherein the selected antibody is an anti-EBV antibody comprising a heavy chain comprising SEQ ID NO: 168 and a light chain comprising SEQ ID NO: 166.

47. A method of claim 2, wherein the selected antibody is an anti-EBV antibody comprising a CDRH1 comprising SEQ ID NO: 248, a CDRH2 comprising SEQ ID NO: 249, a CDRH3 comprising SEQ ID NO: 250, a CDRL1 comprising SEQ ID NO: 251, a CDRL2 comprising SEQ ID NO: 252, and a CDRL3 comprising SEQ ID NO: 253.

48. A method of claim 2, wherein the selected antibody is an anti-HSV antibody comprising HSV8-N and MB66.

49. A method of claim 2, wherein the selected antibody is an anti-*Clostridium difficile* antibody comprising actoxumab or bezlotoxumab.

50. A method of claim 2, wherein the selected antibody is an anti-TNF antibody comprising infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.

51. A method of claim 2, wherein the selected antibody is an anti-TNF antibody comprising a heavy chain comprising SEQ ID NO: 254 and a light chain comprising SEQ ID NO: 255; a CDRH1 comprising SEQ ID NO: 256, a CDRH2 comprising SEQ ID NO: 257, and a CDRH3 comprising SEQ ID NO: 258; a CDRL1 comprising SEQ ID NO: 259, a CDRL2 comprising SEQ ID NO: 260, and a CDRL3 comprising SEQ ID NO: 261; a CDRH1 comprising SEQ ID NO: 262, a CDRH2 comprising SEQ ID NO: 263, and a CDRH3 comprising SEQ ID NO: 264; a CDRL1 comprising SEQ ID NO: 265, a CDRL2 comprising SEQ ID NO: 266, and a CDRL3 comprising SEQ ID NO: 267; a CDRH1 comprising SEQ ID NO: 268, a CDRH2 comprising SEQ ID NO: 269, and a CDRH3 comprising SEQ ID NO: 270; or a CDRL1 comprising SEQ ID NO: 271, a CDRL2 comprising SEQ ID NO: 272, and a CDRL3 comprising SEQ ID NO: 273.

52. A method of claim 2, wherein the genetic modification utilizes a sequence including any of SEQ ID NOs: 87, 88, 89, 90-175, 278-366.

53. A method of claim 2, wherein the B cell is an antibody-producing B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.

54. A B cell modified according to a method of any one of claims 2-53.

55. A B cell of claim 54, wherein the B cell is an antibody-secreting B cell, a memory B cell, a naïve B cell, a B1 B cell or a marginal zone B cell.

56. A method of providing an anti-infection effect in a subject in need thereof comprising administering a therapeutically effective amount of a B cell of claim 54 to the subject thereby providing an anti-infection effect.

57. A method of claim 56, wherein the providing obviates the need for a vaccination.

58. A method of claim 56, wherein the administering replaces a vaccination protocol.

59. A method of claim 56, wherein the subject is immune-suppressed.

60. A method of claim 56, wherein the subject is immune-suppressed as part of a treatment regimen comprising a bone marrow transplant, hematopoietic stem cell transplant, or administration of genetically modified hematopoietic stem cells.

61. A method of providing an anti-inflammatory effect in a subject in need thereof comprising administering a therapeutically effective amount of a B cell of claim 54 to the subject thereby providing an anti-inflammatory effect.

62. A genetic construct for modifying a B cell to express a selected antibody, the genetic construct comprising or encoding (i) a heavy chain promoter, (ii) a signal peptide, (iii) the full length light chain of the selected antibody; (iv) a flexible linker or a skipping element; (v) the variable region of the heavy chain of the selected antibody; and (vi) a splice junction.

63. A genetic construct of claim 62, comprising SEQ ID NOs: 102-175, or 280-289.

64. A genetic construct of claim 62, wherein the flexible linker is between the full length light chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

65. A genetic construct of claim 62, wherein the flexible linker is selected from SEQ ID NOs: 180-184.

66. A genetic construct of claim 62, wherein the flexible linker is a Gly-Ser linker comprising 50-80 amino acids.

67. A genetic construct of claim 62, wherein the flexible linker is a Gly-Ser linker comprises 57 amino acids.

68. A genetic construct of claim 62, wherein the flexible linker is SEQ ID NO: 122.

69. A genetic construct of claim 62, wherein the skipping element is between the full length light

chain of the selected antibody and the variable region of the heavy chain of the selected antibody.

70. A genetic construct of claim 62, wherein the skipping element is a self-cleaving peptide.

71. A genetic construct of claim 70, wherein the self-cleaving peptide is selected from SEQ ID NOs: 176-179.

72. A genetic construct of claim 62, wherein the skipping element is an internal ribosome entry site (IRES).

73. A genetic construct of claim 62, wherein the heavy chain promoter is selected from SEQ ID NOs: 111 and 128.

74. A genetic construct of claim 62, wherein the heavy chain promoter is IgVH1-69 or J558H10.

75. A genetic construct of claim 62, wherein the signal peptide is selected from SEQ ID NOs: 118, 134, and 185-194.

76. A genetic construct of claim 62, wherein the signal peptide is derived from human IgH heavy chain or human IgL light chain.

77. A genetic construct of claim 62, wherein the genetic construct comprises homology arms.

78. A genetic construct of claim 77, wherein the homology arms comprise SEQ ID NOs: 90-101, 110, 125, 127, 140, 142, 143, 153, 170, 171, 173, 174, 278, or 279.

79. A genetic construct of claim 62, wherein the genetic construct encodes a tag.

80. A genetic construct of claim 79, wherein the tag comprises STREPTAG®, STREP® tag II, His tag, Flag tag, Xpress tag, Avi tag, calmodulin tag, polyglutamate tag, HA tag, Myc tag, Nus tag, S tag, SBP tag, Softag 1, Softag 3, or V5 tag.

81. A genetic construct of claim 79, wherein the tag comprises SEQ ID NOs: 122, or 195-204.

82. A genetic construct of claim 62, wherein the selected antibody is an anti-RSV antibody comprising palivizumab, AB1128, or ab20745.

83. A genetic construct of claim 62, wherein the selected antibody is: palivizumab comprising a heavy chain comprising SEQ ID NO: 138 and a light chain comprising SEQ ID NO: 136; palivizumab comprising a heavy chain comprising SEQ ID NO: 138 and a light chain comprising SEQ ID NO: 205; an anti-RSV antibody comprising a heavy chain comprising SEQ ID NO: 123 and a light chain comprising SEQ ID NO: 120; or an anti-RSV antibody comprising a heavy chain comprising SEQ ID NO: 123 and a light chain comprising SEQ ID NO: 206.

84. A genetic construct of claim 62, wherein the selected antibody is an anti-RSV antibody comprising a CDRH1 comprising SEQ ID NO: 207, a CDRH2 comprising SEQ ID NO: 208, a CDRH3 comprising SEQ ID NO: 209; a CDRL1 comprising SEQ ID NO: 210, a CDRL2 comprising SEQ ID NO: 211, and a CDRL3 comprising SEQ ID NO: 212.

85. A genetic construct of claim 62, wherein the selected antibody is an anti-HIV antibody

comprising 10E8, VRC01, ab18633 or 39/5.4A.

86. A genetic construct of claim 62, wherein the selected antibody is an anti-HIV antibody comprising a heavy chain comprising SEQ ID NO: 150 and a light chain comprising SEQ ID NO: 149.

87. A genetic construct of claim 62, wherein the selected antibody is an anti-HIV antibody comprising a CDRH1 comprising SEQ ID NO: 213, a CDRH2 comprising SEQ ID NO: 214, a CDRH3 comprising SEQ ID NO: 215, a CDRL1 comprising SEQ ID NO: 216, a CDRL2 comprising SEQ ID NO: 217, and a CDRL3 comprising SEQ ID NO: 218 or a CDRH1 comprising SEQ ID NO: 219, a CDRH2 comprising SEQ ID NO: 220, a CDRH3 comprising SEQ ID NO: 221, a CDRL1 comprising QYGS, a CDRL2 comprising SGS, and a CDRL3 comprising SEQ ID NO: 222.

88. A genetic construct of claim 62, wherein the selected antibody is an anti-Dengue virus antibody comprising antibody 55, DB2-3, ab155042 or ab80914.

89. A genetic construct of claim 62, wherein the selected antibody is an anti-Dengue virus antibody comprising a CDRH1 comprising SEQ ID NO: 223, a CDRH2 comprising SEQ ID NO: 224, a CDRH3 comprising SEQ ID NO: 225; a CDRL1 comprising SEQ ID NO: 226, a CDRL2 comprising SEQ ID NO: 227, and a CDRKL3 comprising SEQ ID NO: 228 or a CDRH1 comprising SEQ ID NO: 229, a CDRH2 comprising SEQ ID NO: 230, a CDRH3 comprising SEQ ID NO: 231, a CDRL1 comprising SEQ ID NO: 232, a CDRL2 comprising SEQ ID NO: 233, and a CDRL3 comprising SEQ ID NO: 234.

90. A genetic construct of claim 62, wherein the selected antibody is an anti-pertussis antibody comprising a heavy chain comprising SEQ ID NO: 235 and a light chain comprising SEQ ID NO: 236.

91. A genetic construct of claim 62, wherein the selected antibody is an anti-hepatitis C antibody comprising MAB8694 or C7-50.

92. A genetic construct of claim 62, wherein the selected antibody is an anti-hepatitis C antibody comprising a CDRH1 comprising SEQ ID NO: 237, a CDRH2 comprising SEQ ID NO: 238, a CDRH3 comprising SEQ ID NO: 239, a CDRL1 comprising SEQ ID NO: 240, a CDRL2 comprising SEQ ID NO: 241, and a CDRL3 comprising SEQ ID NO: 242.

93. A genetic construct of claim 62, wherein the selected antibody is an anti-influenza virus antibody comprising C102.

94. A genetic construct of claim 62, wherein the selected antibody is an anti-influenza virus antibody comprising a heavy chain comprising SEQ ID NO: 159 and a light chain comprising SEQ ID NO: 158.

95. A genetic construct of claim 62, wherein the selected antibody is an anti-influenza virus

antibody comprising a CDRH1 comprising SEQ ID NO: 243, a CDRH2 comprising SEQ ID NO: 244, a CDRH3 comprising SEQ ID NO: 245, a CDRL1 comprising SEQ ID NO: 246, a CDRL2 comprising KTS, and a CDRL3 comprising SEQ ID NO: 247.

96. A genetic construct of claim 62, wherein the selected antibody is an anti-MPV antibody comprising MPE8.

97. A genetic construct of claim 62, wherein the selected antibody is an anti-CMV antibody comprising MCMV5322A, MCMV3068A, LJP538, or LJP539.

98. A genetic construct of claim 62, wherein the selected antibody is an anti-EBV antibody comprising a heavy chain comprising SEQ ID NO: 168 and a light chain comprising SEQ ID NO: 166.

99. A genetic construct of claim 62, wherein the selected antibody is an anti-EBV antibody comprising a CDRH1 comprising SEQ ID NO: 248, a CDRH2 comprising SEQ ID NO: 249, a CDRH3 comprising SEQ ID NO: 250, a CDRL1 comprising SEQ ID NO: 251, a CDRL2 comprising SEQ ID NO: 252, and a CDRL3 comprising SEQ ID NO: 253.

100.A genetic construct of claim 62, wherein the selected antibody is an anti-HSV antibody comprising HSV8-N and MB66.

101.A genetic construct of claim 62, wherein the selected antibody is an anti-*Clostridium difficile* antibody comprising actoxumab or bezlotoxumab.

102.A genetic construct of claim 62, wherein the selected antibody is an anti-TNF antibody comprising infliximab, adalimumab, etanercept, certolizumab, or accepted biosimilars thereof.

103.A genetic construct of claim 62, wherein the selected antibody is an anti-TNF antibody comprising a heavy chain comprising SEQ ID NO: 254 and a light chain comprising SEQ ID NO: 255; a CDRH1 comprising SEQ ID NO: 256, a CDRH2 comprising SEQ ID NO: 257, and a CDRH3 comprising SEQ ID NO: 258; a CDRL1 comprising SEQ ID NO: 259, a CDRL2 comprising SEQ ID NO: 260, and a CDRL3 comprising SEQ ID NO: 261; a CDRH1 comprising SEQ ID NO: 262, a CDRH2 comprising SEQ ID NO: 263, and a CDRH3 comprising SEQ ID NO: 264; a CDRL1 comprising SEQ ID NO: 265, a CDRL2 comprising SEQ ID NO: 266, and a CDRL3 comprising SEQ ID NO: 267; a CDRH1 comprising SEQ ID NO: 268, a CDRH2 comprising SEQ ID NO: 269, and a CDRH3 comprising SEQ ID NO: 270; or a CDRL1 comprising SEQ ID NO: 271, a CDRL2 comprising SEQ ID NO: 272, and a CDRL3 comprising SEQ ID NO: 273.

104.A kit for genetically modifying a B cell comprising a genetic construct of any of claims 62-103 and a gRNA targeting SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4.

105.A kit of claim 104, wherein the gRNA is selected from one or more of SEQ ID NOs: 87, 88, 89, and 290-366.

106.A kit of claim 104, further comprising a nuclease.

107.A kit of claim 106, wherein the nuclease is Cas9 or Cpf1.

108.A kit of claim 104, further comprising a nanoparticle or adeno-associated viral vector.

109.A kit of claim 106, wherein the gRNA and nuclease are associated with a nanoparticle.

110.A kit of claim 104, wherein the genetic construct is part of an adeno-associated viral vector.