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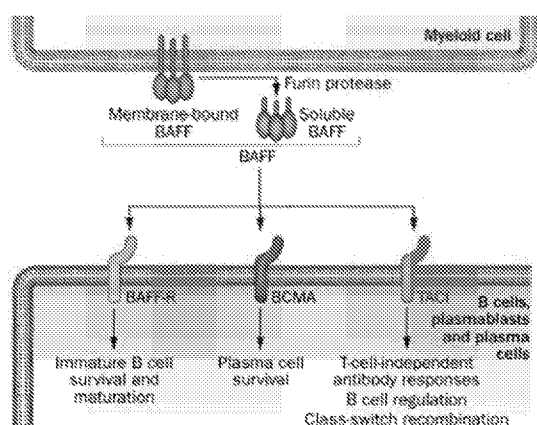


FIG. 1

(57) Abstract: This disclosure provides a high-throughput method for identifying and isolating a candidate BAFT receptor antagonist and methods for their use in vitro and in vivo.



CYCLOTIDE-BASED POLYPEPTIDES FOR THERAPEUTIC TARGETING OF BAFF RECEPTORS IN SLE

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/691,564, filed June 28, 2018, the content of which is hereby incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with a sponsor under the Grant Nos. R01 GM090323 and R01 GM113363, awarded by the National Institute for Health (NIH). The U.S. Government has certain rights to the invention.

BACKGROUND

[0003] Current therapies for systemic lupus erythematosus (“SLE”) commonly referred to as lupus, are based mainly on the use of non-specific immunosuppressive drugs that cause serious toxic side effects. This highlights the urgent unmet need for novel therapeutic means with improved efficacy and lower toxicity. Although the pathogenesis of SLE still remains incompletely understood, the prevalence of autoantibodies early on, even before clinical symptoms of SLE are found, implicates B cell dysregulation as a contributing factor to disease. Accordingly, several B-cell targeted treatments are starting to emerge. These include the use of monoclonal antibodies and soluble receptors for antagonizing BAFF (B-cell-activating factor).

[0004] B cells play a central role in SLE pathogenesis, and therapeutic targeting of B cells has emerged as an attractive approach. BAFF (B-cell-activating factor) is a critical survival factor for transitional and mature B cells and is a proven therapeutic target for SLE. The first (and, so far, the only) BAFF antagonist approved by the FDA is belimumab, a human IgG mAb that binds and neutralizes soluble BAFF. However, belimumab has demonstrated only modest efficacy for the treatment of SLE in two independent clinical trials. Thus, a need exists in the art for alternative, effective therapies. This disclosure satisfies that need and provides related advantages as well.

SUMMARY

[0005] As disclosed herein, Applicant presents an alternative approach for effective BAFF antagonist therapy, i.e. to target the BAFF receptor rather than soluble BAFF. In one aspect, provided herein are selective antagonists for specific pairs (BR3/TACI or BR3/BCMA) of BAFF receptors. Applicant developed unique cyclotide-based molecular scaffolds for generating genetically-encoded molecular libraries can be screened and selected for the ability to selectively antagonize the interaction between soluble BAFF and BR3 and BCMA (but not TACI) or BR3 and TACI (but not BCMA).

[0006] Cyclotides are a family of large plant-derived backbone-cyclized polypeptides (≈ 30 amino acids long) that share a 3' disulfide-stabilized core characterized by an unusual knotted structure. They have several characteristics that make them ideal drug development tools. They are remarkably stable to chemical, thermal and proteolytic degradation due to the cyclic topology and cysteine knot. They are small, making them readily accessible by chemical synthesis. Cyclotides can be encoded within standard cloning vectors, expressed in bacteria or animal cells, and are amenable to substantial sequence variation. Cyclotides can be also engineered to target intracellular and extracellular protein interactions. Cyclotides have been shown to be orally bioavailable. However, the cyclotide approach has never, to the best of Applicant's knowledge, been applied to selectively antagonize the interaction between soluble BAFF and BR3, and (but not TACI) or BR3 and TACI (but not BCMA).

[0007] Thus, in one aspect, the disclosure provides an isolated peptide comprising, or alternatively consisting essentially of, or yet further consisting of a protease recognition leading signal, fused or grafted to a cyclotide, optionally comprising a linker and a candidate BAFF receptor antagonist. In one embodiment, the candidate BAFF receptor antagonist comprises a CDR of an antibody that specifically binds a BAFF receptor. Also provided are first reporter polypeptides comprising a BAFF ligand fused to a reporter element. Further provided are second BAFF receptor polypeptides that bind to the BAFF ligand fused to a reporter element that when in contact with the first reporter polypeptide, emit a detectable signal.

[0008] Non-limiting examples, peptides for use as a linker comprises, or alternatively consists essentially of, or yet further consists of a peptide selected from the group consisting

of GXXGXP, wherein X is any amino acid; GGSGGF; GASGPG; GSGAPG; ASKAPG; and ASRAPG.

[0009] Also provided by this disclosure are isolated polynucleotides encoding the polypeptides described above or a complement thereof. The polynucleotides can be incorporated and operatively linked to an expression or replication vector, which can further comprise the appropriate regulatory sequences for expression or duplication of the sequences, that in one aspect are inducible and distinct. The polynucleotides and vectors can further comprise a label or tag, e.g., a fluorescent label or tag.

[0010] The peptides, polynucleotides, vectors can be incorporated into host cells, such as a prokaryotic (*E. coli*) or eukaryotic cells.

[0011] The peptides, polynucleotides, vectors, host cells can be combined with a carrier, such as a pharmaceutically acceptable carrier.

[0012] The peptides and compositions can be used to identify candidate BAFF receptor antagonists, as described herein. The methods can be performed in vitro (cell free) or in a cellular environment. Further provided are the candidate agents identified by this method.

[0013] In a yet further aspect, a kit is provided that comprises, or alternatively consists essentially of, or yet further consisting of, any of one or more of the isolated polypeptide as disclosed above, an isolated polynucleotide as disclosed herein, an isolated vector or host cell as described above, or the compositions, and instructions for use.

[0014] The methods as disclosed herein are for the isolation and characterization of BAFF-specific antagonists. In one aspect, cell-based libraries (*E. coli* cell libraries) can be used where every single cell expresses a different cyclotide in a single cell-single compound approach. These compounds are screened and selected in high throughput fashion for their ability to inhibit the corresponding BAFF-receptor interaction inside the bacterial cell using a genetically-encoded fluorogenic reporter in combination with fluorescence-activated cell sorting (FACS). Selected cyclotides are then characterized in vitro and in vivo in a murine SLE model.

[0015] To screen and select cyclotide-based peptides that disrupt BAFF/BR3 and BAFF/BCMA interactions (but not BAFF/TACI interactions) or that disrupt BAFF/BR3 and

BAFF/TACI interactions (but not BAFF/BCMA interactions), large genetically-encoded libraries of cyclotides in living *E. coli* cells ($\approx 10^9$) are produced. In-cell FRET-based screening reporters can be used to select cyclotides comprising antagonists able to inhibit BAFF binding to its individual receptors.

[0016] Selected cyclotides encoding candidate receptor antagonists are tested *in vitro* using a combination of fluorescence assays and nuclear magnetic resonance (NMR). Biological activity is assayed using cell lines expressing the different individual BAFF receptors and primary murine B cells derived from genetically-engineered mice that express only one of the three BAFF receptors.

[0017] The selected cyclotides are also evaluated for *in vivo* activity. The cyclotides with the best *in vitro* activity are evaluated for biologic and therapeutic activities *in vivo* using SLE-prone NZM 2328 mice.

[0018] Further provided by this disclosure are the cyclotides prepared by the method, that in one aspect demonstrate *in vitro* and/or *in vivo* BAFF selective activity.

[0019] This disclosure provides a polypeptide comprising, or consisting essentially of, or yet further consisting of the disclosed cyclotide polypeptides, and equivalents thereof, which retain the ability to inhibit the interaction between BAFF and its receptor BR3. Further provided are polynucleotides encoding the polypeptides, and their complements as well as vectors and host cells comprising the polynucleotides, as well as methods for recombinant

[0020] Methods for use of the cyclotides and polypeptides contained within the cyclotides are further provided.

BRIEF DESCRIPTION OF DRAWINGS

[0021] FIG. 1: Soluble BAFF signaling and the corresponding cellular effects on B cells, plasmablasts and plasma cells. Figure modified from reference [1].

[0022] FIG. 2: Primary and tertiary structures of cyclotides from the Möbius (kalata B1), bracelet (cycloviolacin O1) and trypsin inhibitor (MCoTI-II) subfamilies. Conserved cysteine residues are marked in yellow and disulfide connectivities in red. The circular backbone topology is shown with a line.

[0023] FIGS. 3A-3B: Clinical disease and renal immunopathology among NZM mice deficient in two BAFF receptors. **FIG. 3A:** WT (n = 35, black lines), BCMA/TACI (n = 34, red lines), BR3/TACI (n = 36, gray lines), and BR3/BCMA (n = 36, lines) mice were monitored for 12 months for development of severe proteinuria (left) and survival (right). Data are plotted as the fraction of mice over time that did not develop severe proteinuria (left) or remained alive (right). **FIG. 3B** Kidney sections from 6- to 7-month-old NZM WT, BCMA/TACI, BR3/TACI, and BR3/BCMA mice were stained for IgG immunofluorescence (top row) or C3 immunofluorescence (middle row) or with hematoxylin and eosin (H&E) for histologic evaluation. Original magnification 200 X.

[0024] FIGS. 4A-4B: Genetically-encoded libraries based on the MCoTI cyclotide scaffold. **FIG. 4A:** Molecular diversity of libraries based on loop 2, loop 5 and loops 2 & 5 of MCoTII. **FIG. 4B:** E. coli expression of selected members of the MCoTI cyclotide library based on loop 2. Cyclotides were captured with trypsin (and therefore correctly folded) and characterized by ES-MS.

[0025] FIG. 5: Principle for the cell-based screening of biosynthesized genetically-encoded cyclotide libraries using a FRET-based approach. Protein domains A refers to soluble BAFF and B refers to the corresponding Cys-rich domains of receptors BR3, TACI or BCMA.

[0026] FIGS. 6A-6C: Cell-based FRET reporter to screen for RING-mediated Hdm2-HdmX interactions as depicted in FIG. 5. **FIG. 6A:** Coexpression of several RING domains fused to fluorescent proteins YPet and CyPet. **FIG. 6B:** FACS analysis of cells with FRET-ON and FRET-OFF phenotypes. **FIG. 6C:** Enrichment of FRET-OFF phenotype by performing multiple rounds of FACS.

DETAILED DESCRIPTION

[0027] Before the compositions and methods are described, it is to be understood that the invention is not limited to the particular methodologies, protocols, cell lines, assays, and reagents described, as these may vary. It is also to be understood that the terminology used herein is intended to describe particular embodiments of the present invention, and is in no way intended to limit the scope of the present invention as set forth in the appended claims.

[0028] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel et al. eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson et al. (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson et al. (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Herzenberg et al. eds (1996) *Weir's Handbook of Experimental Immunology; Manipulating the Mouse Embryo: A Laboratory Manual*, 3rd edition (Cold Spring Harbor Laboratory Press (2002)); *Current Protocols In Molecular Biology* (F. M. Ausubel, et al. eds., (1987)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)); Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*; Harlow and Lane, eds. (1999) *Using Antibodies, A Laboratory Manual*; *Animal Cell Culture* (R.I. Freshney, ed. (1987)); Zigova, Sanberg and Sanchez-Ramos, eds. (2002) *Neural Stem Cells*.

[0029] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 0.1 or 1 where appropriate. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term “about”. The term “about” also includes the exact value “X” in addition to minor increments of “X” such as “X + 0.1 or 1” or “X – 0.1 or 1,” where appropriate. It also is to be understood, although not

always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[0030] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, etc. As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, etc. As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above.

[0031] Throughout and within this disclosure the patent and technical literature is identified by a bibliographic citation or by a Arabic number. The bibliographic citations for these references are found in this disclosure immediately preceding the claims. All references disclosed here are incorporated by reference to more fully describe the state of the art to which this invention pertains.

Definitions

[0032] As used in the specification and claims, the singular form “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

[0033] As used herein, the term “comprising” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention or process steps to produce a composition or achieve an

intended result. Embodiments defined by each of these transition terms are within the scope of this invention.

[0034] The term “isolated” as used herein with respect to cells, nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. The term “isolated” as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to cells or polypeptides which are isolated from other cellular proteins or tissues. Isolated polypeptides are meant to encompass both purified and recombinant polypeptides.

[0035] As used herein, the term “recombinant” as it pertains to polypeptides or polynucleotides intends a form of the polypeptide or polynucleotide that does not exist naturally, a non-limiting example of which can be created by combining polynucleotides or polypeptides that would not normally occur together.

[0036] A “subject,” “individual” or “patient” is used interchangeably herein and refers to a vertebrate, for example a primate, a mammal or preferably a human. Mammals include, but are not limited to equines, canines, bovines, ovines, murines, rats, simians, humans, farm animals, sport animals and pets.

[0037] “Cells,” “host cells” or “recombinant host cells” are terms used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0038] “Amplify” “amplifying” or “amplification” of a polynucleotide sequence includes methods such as traditional cloning methodologies, PCR, ligation amplification (or ligase chain reaction, LCR) or other amplification methods. These methods are known and practiced in the art. See, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202 and Innis et al.

(1990) Mol. Cell Biol. 10(11):5977-5982 (for PCR); and Wu et al. (1989) Genomics 4:560-569 (for LCR). In general, the PCR procedure describes a method of gene amplification which is comprised of (i) sequence-specific hybridization of primers to specific genes within a DNA sample (or library), (ii) subsequent amplification involving multiple rounds of annealing, elongation, and denaturation using a DNA polymerase, and (iii) screening the PCR products for a band of the correct size. The primers used are oligonucleotides of sufficient length and appropriate sequence to provide initiation of polymerization, i.e. each primer is specifically designed to be complementary to each strand of the genomic locus to be amplified.

[0039] Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from a particular region are preferably complementary to, and hybridize specifically to sequences in the target region or in its flanking regions. Nucleic acid sequences generated by amplification may be sequenced directly. Alternatively the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments is known in the art.

[0040] As used herein, the term “grafted” intends replaced or inserted, e.g., the phrase “grafted between” means that a peptide replaces the amino acid residues between the two indicated amino acids.

[0041] As used herein, the term “fused” intends the attachment of two amino acids to each other by a peptide bond.

[0042] The term “genotype” refers to the specific allelic composition of an entire cell, a certain gene or a specific polynucleotide region of a genome, whereas the term “phenotype” refers to the detectable outward manifestations of a specific genotype.

[0043] As used herein, the term “gene” or “recombinant gene” refers to a nucleic acid molecule comprising an open reading frame and including at least one exon and (optionally) an intron sequence. A gene may also refer to a polymorphic or a mutant form or allele of a gene.

[0044] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present invention.

[0045] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 98 % or 99 %) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Ausubel et al. eds. (2007) Current Protocols in Molecular Biology. Preferably, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ + PDB + GenBank CDS translations + SwissProtein + SPupdate + PIR. Details of these programs can be found at the following Internet address:

<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>, last accessed on May 21, 2008. Biologically equivalent polynucleotides are those having the above-noted specified percent homology and encoding a polypeptide having the same or similar biological activity.

[0046] The term “an equivalent” nucleic acid, polynucleotide or peptide refers to a nucleic acid having a nucleotide sequence having a certain degree of homology with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof.

[0047] "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogsteen binding, or in any other sequence-specific manner. The complex may comprise two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PC reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[0048] Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6x SSC to about 10x SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4x SSC to about 8x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9x SSC to about 2x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5x SSC to about 2x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about 1x SSC to about 0.1x SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1x SSC, 0.1x SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

[0049] As used herein, the term "oligonucleotide" refers to polynucleotides such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term should also be understood to include, as equivalents, derivatives, variants and analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides. Deoxyribonucleotides include deoxyadenosine, deoxycytidine, deoxyguanosine, and deoxythymidine. For purposes of clarity, when referring herein to a nucleotide of a nucleic acid, which can be DNA or an RNA, the terms "adenosine", "cytidine", "guanosine", and "thymidine" are used. It is understood that if the nucleic acid is RNA, a nucleotide having a uracil base is uridine.

[0050] The terms “polynucleotide” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this invention that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0051] A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. The term “polymorphism” refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, i.e., two different nucleotide sequences, is referred to as a “polymorphic region of a gene”. A polymorphic region can be a single nucleotide, the identity of which differs in different alleles.

[0052] “Overexpression” or “underexpression” refers to increased or decreased expression, or alternatively a differential expression, of a gene in a test sample as compared to the expression level of that gene in the control sample. In one aspect, the test sample is a diseased cell, and the control sample is a normal cell. In another aspect, the test sample is an

experimentally manipulated or biologically altered cell, and the control sample is the cell prior to the experimental manipulation or biological alteration. In yet another aspect, the test sample is a sample from a patient, and the control sample is a similar sample from a healthy individual. In a yet further aspect, the test sample is a sample from a patient and the control sample is a similar sample from patient not having the desired clinical outcome. In one aspect, the differential expression is about 1.5 times, or alternatively, about 2.0 times, or alternatively, about 2.0 times, or alternatively, about 3.0 times, or alternatively, about 5 times, or alternatively, about 10 times, or alternatively about 50 times, or yet further alternatively more than about 100 times higher or lower than the expression level detected in the control sample. Alternatively, the gene is referred to as “over expressed” or “under expressed”. Alternatively, the gene may also be referred to as “up regulated” or “down regulated.”

[0053] As used herein, the term “carrier” encompasses any of the standard carriers, such as a phosphate buffered saline solution, buffers, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Sambrook and Russell (2001), *supra*. Those skilled in the art will know many other suitable carriers for binding polynucleotides, or will be able to ascertain the same by use of routine experimentation. In one aspect of the invention, the carrier is a buffered solution such as, but not limited to, a PCR buffer solution.

[0054] A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins; polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

[0055] “Gene delivery,” “gene transfer,” and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a “transgene”) into a host cell, irrespective of the method used for the introduction. Such methods include a

variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection, sometimes called transduction), transfection, transformation or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of “naked” polynucleotides (such as electroporation, “gene gun” delivery and various other techniques used for the introduction of polynucleotides). Unless otherwise specified, the term transfected, transduced or transformed may be used interchangeably herein to indicate the presence of exogenous polynucleotides or the expressed polypeptide therefrom in a cell. The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

[0056] A cell that “stably expresses” an exogenous polypeptide is one that continues to express a polypeptide encoded by an exogenous gene introduced into the cell either after replication if the cell is dividing or for longer than a day, up to about a week, up to about two weeks, up to three weeks, up to four weeks, for several weeks, up to a month, up to two months, up to three months, for several months, up to a year or more.

[0057] The term “express” refers to the production of a gene product.

[0058] As used herein, “expression” refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in an eukaryotic cell.

[0059] A “gene product” or alternatively a “gene expression product” refers to the amino acid (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

[0060] “Under transcriptional control” is a term well understood in the art and indicates that transcription of a polynucleotide sequence, usually a DNA sequence, depends on its being operatively linked to an element which contributes to the initiation of, or promotes, transcription. “Operatively linked” intends the polynucleotides are arranged in a manner that allows them to function in a cell.

[0061] The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[0062] As used herein, a “vector” is a vehicle for transferring genetic material into a cell. Examples of such include, but are not limited to plasmids and viral vectors. A viral vector is a virus that has been modified to transduce genetic material into a cell. A plasmid vector is made by splicing a DNA construct into a plasmid. As is apparent to those of skill in the art, the appropriate regulatory elements are included in the vectors to guide replication and/or expression of the genetic material in the selected host cell.

[0063] A “viral vector” is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either in vivo, ex vivo or in vitro. Examples of viral vectors include retroviral vectors, lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger and Dubensky (1999) *Curr. Opin. Biotechnol.* 5:434-439 and Ying et al. (1999) *Nat. Med.* 5(7):823-827.

[0064] In aspects where gene transfer is mediated by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene. As used herein, “retroviral mediated gene transfer” or “retroviral transduction” carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. Retroviruses carry their genetic information in the form of RNA; however, once the virus infects a cell, the RNA is reverse-transcribed into the DNA form which integrates into the genomic DNA of the infected cell. The integrated DNA form is called a provirus. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

A “lentiviral vector” is a type of retroviral vector well-known in the art that has certain advantages in transducing nondividing cells as compared to other retroviral vectors. See, Trono D. (2002) *Lentiviral Vectors*, New York: Spring-Verlag Berlin Heidelberg.

[0065] In aspects where gene transfer is mediated by a DNA viral vector, such as an adenovirus (Ad) or adeno-associated virus (AAV), a vector construct refers to the polynucleotide comprising the viral genome or part thereof, and a transgene. Adenoviruses (Ads) are a relatively well characterized, homogenous group of viruses, including over 50 serotypes. See, e.g., International PCT Application No. WO 95/27071. Ads do not require integration into the host cell genome. Recombinant Ad derived vectors, particularly those that reduce the potential for recombination and generation of wild-type virus, have also been constructed. See, International PCT Application Nos. WO 95/00655 and WO 95/11984. Wild-type AAV has high infectivity and specificity integrating into the host cell's genome. See, Hermonat and Muzyczka (1984) *Proc. Natl. Acad. Sci. USA* 81:6466-6470 and Lebkowski et al. (1988) *Mol. Cell. Biol.* 8:3988-3996.

[0066] Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA in vitro or in vivo, and are commercially available from sources such as Stratagene (La Jolla, CA) and Promega Biotech (Madison, WI). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' and/or 3' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites can be inserted immediately 5' of the start codon to enhance expression.

[0067] Gene delivery vehicles also include several non-viral vectors, including DNA/liposome complexes, and targeted viral protein-DNA complexes. Liposomes that also comprise a targeting antibody or fragment thereof can be used in the methods of this invention. To enhance delivery to a cell, the nucleic acid or proteins of this invention can be conjugated to antibodies or binding fragments thereof which bind cell surface antigens, e.g., a cell surface marker found on stem cells.

[0068] A “plasmid” is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances.

[0069] “Plasmids” used in genetic engineering are called “plasmic vectors”. Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacteria produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene. This is a cheap and easy way of mass-producing a gene or the protein it then codes for.

[0070] “Eukaryotic cells” comprise all of the life kingdoms except monera. They can be easily distinguished through a membrane-bound nucleus. Animals, plants, fungi, and protists are eukaryotes or organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane-bound structure is the nucleus. A eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Non-limiting examples include simian, bovine, ovine, porcine, murine, rats, canine, equine, feline, avian, reptilian and human.

[0071] “Prokaryotic cells” that usually lack a nucleus or any other membrane-bound organelles and are divided into two domains, bacteria and archaea. Additionally, instead of having chromosomal DNA, these cells’ genetic information is in a circular loop called a plasmid. Bacterial cells are very small, roughly the size of an animal mitochondrion (about 1-2 μm in diameter and 10 μm long). Prokaryotic cells feature three major shapes: rod shaped, spherical, and spiral. Instead of going through elaborate replication processes like

eukaryotes, bacterial cells divide by binary fission. Examples include but are not limited to prokaryotic Cyanobacteria, *bacillus* bacteria, *E. coli* bacterium, and *Salmonella* bacterium.

[0072] The term “propagate” means to grow a cell or population of cells. The term “growing” also refers to the proliferation of cells in the presence of supporting media, nutrients, growth factors, support cells, or any chemical or biological compound necessary for obtaining the desired number of cells or cell type.

[0073] The term “culturing” refers to the *in vitro* propagation of cells or organisms on or in media of various kinds. It is understood that the descendants of a cell grown in culture may not be completely identical (i.e., morphologically, genetically, or phenotypically) to the parent cell.

[0074] A “probe” when used in the context of polynucleotide manipulation refers to an oligonucleotide that is provided as a reagent to detect a target potentially present in a sample of interest by hybridizing with the target. Usually, a probe will comprise a label or a means by which a label can be attached, either before or subsequent to the hybridization reaction. Suitable labels are described and exemplified herein.

[0075] A “primer” is a short polynucleotide, generally with a free 3’ -OH group that binds to a target or “template” potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. A “polymerase chain reaction” (“PCR”) is a reaction in which replicate copies are made of a target polynucleotide using a “pair of primers” or a “set of primers” consisting of an “upstream” and a “downstream” primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and taught, for example in MacPherson et al. (1991) PCR: A Practical Approach, IRL Press at Oxford University Press. All processes of producing replicate copies of a polynucleotide, such as PCR or gene cloning, are collectively referred to herein as “replication.” A primer can also be used as a probe in hybridization reactions, such as Southern or Northern blot analyses. Sambrook et al., *supra*. The primers may optionally contain detectable labels and are exemplified and described herein.

[0076] As used herein, the term “detectable label” intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be

detected, e.g., polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component.

[0077] Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

[0078] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue.TM., and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

[0079] In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a

cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

[0080] Attachment of the fluorescent label may be either directly to the cellular component or compound or alternatively, can be via a linker. Suitable binding pairs for use in indirectly linking the fluorescent label to the intermediate include, but are not limited to, antigens/antibodies, e.g., rhodamine/anti-rhodamine, biotin/avidin and biotin/streptavidin.

[0081] As used herein, the term “discrete marker” intends an entity or molecule that allows for the selective identification of a peptide of interest. Non-limiting examples of such include, isotope-coded affinity tags, isobaric tags, radioactively labeled amino acids, and bioorthogonal noncanonical amino acid tags. Dieterich et al. (2006) PNAS, 103(25):9482-9487.

[0082] The phrase “solid support” refers to non-aqueous surfaces such as “culture plates” “gene chips” or “microarrays.” Gene chips or microarrays can be used for diagnostic and therapeutic purposes by a number of techniques known to one of skill in the art. In one technique, oligonucleotides are attached and arrayed on a gene chip for determining the DNA sequence by the hybridization approach, such as that outlined in U.S. Patent Nos. 6,025,136 and 6,018,041. Polynucleotides and probes can be used for detection of a genetic sequence. Such techniques have been described, for example, in U.S. Patent Nos. 5,968,740 and 5,858,659. A probe also can be attached or affixed to an electrode surface for the electrochemical detection of nucleic acid sequences such as described by Kayem et al. U.S. Patent No. 5,952,172 and by Kelley et al. (1999) Nucleic Acids Res. 27:4830-4837.

[0083] Various “gene chips” or “microarrays” and similar technologies are known in the art. Examples of such include, but are not limited to, LabCard (ACLARA Bio Sciences Inc.); GeneChip (Affymetric, Inc); LabChip (Caliper Technologies Corp); a low-density array with electrochemical sensing (Clinical Micro Sensors); LabCD System (Gamera Bioscience Corp.); Omni Grid (Gene Machines); Q Array (Genetix Ltd.); a high-throughput, automated mass spectrometry systems with liquid-phase expression technology (Gene Trace Systems,

Inc.); a thermal jet spotting system (Hewlett Packard Company); Hyseq HyChip (Hyseq, Inc.); BeadArray (Illumina, Inc.); GEM (Incyte Microarray Systems); a high-throughput microarray system that can dispense from 12 to 64 spots onto multiple glass slides (Intelligent Bio-Instruments); Molecular Biology Workstation and NanoChip (Nanogen, Inc.); a microfluidic glass chip (Orchid Biosciences, Inc.); BioChip Arrayer with four PiezoTip piezoelectric drop-on-demand tips (Packard Instruments, Inc.); FlexJet (Rosetta Inpharmatic, Inc.); MALDI-TOF mass spectrometer (Sequenome); ChipMaker 2 and ChipMaker 3 (TeleChem International, Inc.); and GenoSensor (Vysis, Inc.) as identified and described in Heller (2002) *Annu. Rev. Biomed. Eng.* 4:129-153. Examples of “gene chips” or a “microarrays” are also described in U.S. Patent Publication Nos. 2007/0111322; 2007/0099198; 2007/0084997; 2007/0059769 and 2007/0059765 and U.S. Patent Nos.: 7,138,506; 7,070,740 and 6,989,267.

[0084] In one aspect, “gene chips” or “microarrays” containing probes or primers homologous to p53 are prepared. A suitable sample is obtained from the patient, extraction of genomic DNA, RNA, protein or any combination thereof is conducted and amplified if necessary. The sample is contacted to the gene chip or microarray panel under conditions suitable for hybridization of the gene(s) or gene product(s) of interest to the probe(s) or primer(s) contained on the gene chip or microarray. The probes or primers may be detectably labeled thereby identifying the sequence(s) of interest. Alternatively, a chemical or biological reaction may be used to identify the probes or primers which hybridized with the DNA or RNA of the gene(s) of interest. The genotypes or phenotype of the patient is then determined with the aid of the aforementioned apparatus and methods.

[0085] A “composition” is intended to mean a combination of active agent and another compound or composition, inert (for example, a detectable agent or label) or active, such as an adjuvant.

[0086] A “pharmaceutical composition” is intended to include the combination of an active agent with a carrier, inert or active, making the composition suitable for diagnostic or therapeutic use in vitro, in vivo or ex vivo.

[0087] As used herein, the term “pharmaceutically acceptable carrier” encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and

emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin (1975) Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton).

[0088] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including the activity of the specific compound employed, bioavailability of the compound, the route of administration, the age of the animal and its body weight, general health, sex, the diet of the animal, the time of administration, the rate of excretion, the drug combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from in vitro and/or in vivo tests initially can provide useful guidance on the proper doses for patient administration. Studies in animal models generally may be used for guidance regarding effective dosages for treatment of diseases. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective in vitro. Thus, where a compound is found to demonstrate in vitro activity, for example as noted in the Tables discussed below one can extrapolate to an effective dosage for administration in vivo. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks. Consistent with this definition and as used herein, the term “therapeutically effective amount” is an amount sufficient to treat a specified disorder or disease or alternatively to obtain a pharmacological response treating a cancer.

[0089] As used herein, “treating” or “treatment” of a disease in a patient refers to (1) preventing the symptoms or disease from occurring in an animal that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease or the symptoms of the

disease. As understood in the art, “treatment” is an approach for obtaining beneficial or desired results, including clinical results. For the purposes of this invention, beneficial or desired results can include one or more, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of a condition (including a disease), stabilized (i.e., not worsening) state of a condition (including disease), delay or slowing of condition (including disease), progression, amelioration or palliation of the condition (including disease), states and remission (whether partial or total), whether detectable or undetectable. Preferred are compounds that are potent and can be administered locally at very low doses, thus minimizing systemic adverse effects.

[0090] A “control” is an alternative subject or sample used in an experiment for comparison purpose. A control can be “positive” or “negative”. For example, where the purpose of the experiment is to determine a correlation of a mutated allele with a particular phenotype, it is generally preferable to use a positive control (a sample from a subject, carrying such mutation and exhibiting the desired phenotype), and a negative control (a subject or a sample from a subject lacking the mutated allele and lacking the phenotype).

[0091] As used herein, the term “intein” intends a segment of a protein that is able to excise itself and rejoin the remaining portions (the exteins) with a peptide bond by protein splicing. Inteins have also been called “protein introns.” Inteins are known in the art and sequences are publicly available, see for example, InBase, The Intein Database and Registry, available at the web address: neb.com/external-links/inbase (accessed on March 5, 2013) and Inteins – protein introns, available at the web address: bioinformatics.weizmann.ac.il/~pietro/inteins/ (accessed on March 5, 2013).

Modes for Carrying Out the Disclosure

[0092] *BAFF and its receptors as therapeutic targets in SLE.* BAFF, a member of the TNF ligand superfamily, is a 285-amino acid type-II transmembrane precursor protein that is cleaved by a furin protease to yield a soluble, biologically active ligand [6] that binds to three receptors, BCMA, TACI and BR3 (also known as BAFF-R) on the surface of B cells (FIG. 1) [7-9]. BAFF is essential for B cell survival and maturation [10-12] and contributes importantly to the differentiation of immature B cells [13] and to Ig class switching and Ig production [14]. Given the importance of B cells to SLE, it is not surprising that BAFF has

become a therapeutic target in this disorder [2]. Indeed, the anti-BAFF mAb belimumab was recently approved by the FDA for the treatment of SLE. However, belimumab demonstrated only modest efficacy in two independent clinical trials [4, 5, 15]. Given the limited therapeutic capacity effected through global neutralization of BAFF elimination/neutralization strategy, the possibility that selective targeting of specific BAFF receptors could augment efficacy needs to be seriously entertained.

[0093] Expression of the three BAFF receptors is neither uniform nor constant as B cells mature and differentiate [7-9]. For example, neither pro-B nor pre-B cells isolated from bone marrow express BR3, whereas BR3 becomes expressed on immature B cells as they acquire a functional B-cell antigen receptor [16]. In contrast, BCMA is, at most, minimally detectable on non-terminally differentiated mature B cells, whereas it becomes significantly upregulated as the B cells terminally differentiate [17, 18].

[0094] Genetic depletion of the individual BAFF receptors results in strikingly divergent phenotypes. BCMA-deficient mice display a near-normal phenotype. These mice harbor normal numbers and subsets of lymphocytes and do not manifest any signs of immunodeficiency *in vivo* [19, 20], although they do not maintain as many antigen-specific long-lived Ig-secreting cells in the bone marrow following immunization as BCMA-sufficient mice do [21].

[0095] In contrast to the relatively benign and simple phenotype of BCMA-deficient mice, the phenotype of TACI-deficient mice is highly complex, with elements of immunodeficiency and autoimmunity. These mice mount impaired antibody responses to T cell-independent antigens but, at the same time, harbor increased number of T helper and B cells. Indeed, as TACI-deficient mice age, they develop circulating autoantibodies, Ig deposition in their kidneys, and histological glomerulonephritis and premature death [22-24].

[0096] Among mice deficient in a single BAFF receptor, it is only BR3-deficient mice that manifest deficiencies in mature B cells and antibody responses to degrees similar to those manifest by BAFF-deficient mice [25, 26]. For example, B cells do not expand in BR3-deficient mice after treatment with exogenous BAFF. In addition, B cells bearing a mutated *BR3* gene also display decreased *in vivo* survival.

[0097] With these observations in non-autoimmune-prone mice as a backdrop, the Stohl and Jacob laboratories reasoned that genetic ablation of the *Br3* gene in SLE-prone NZM 2328 mice (hereafter called NZM mice), by virtue of the anticipated marked diminution in numbers of B cells, would markedly attenuate development of SLE. In contrast, genetic ablation of the *Taci* gene, by virtue of the anticipated expansion in B cell numbers, would accelerate development of SLE. Remarkably, neither of these outcomes came to pass. NZM mice singly-deficient in any of the BAFF receptors (i.e., NZM.*Br3*^{-/-} mice, NZM.*Taci*^{-/-} mice, and NZM.*Bcma*^{-/-} mice), despite manifesting their expected dramatic differences in B cell profiles, all developed clinical disease with time courses indistinguishable from that of NZM wild-type (WT mice) [27]. That is, any therapeutic approach that eliminates (or, presumably, neutralizes) only any single BAFF receptor is not efficacious in SLE-prone NZM mice and would likely not be efficacious in human SLE as well. This surprising result prompted additional experiments, described herein.

Targeting protein-protein interactions with cyclotides

[0098] The success of protein-based therapeutics is revolutionizing drug development. Unlike small molecule drugs, peptide and protein-based therapeutics can target with high selectivity and specificity defective protein-protein interactions involved in human disease. Despite their success, however, there are still numerous stability and delivery issues associated with their use as therapeutic agents. For example, monoclonal antibodies, one the most successful protein-based therapeutics with several blockbuster drugs on the market and many more in clinical development, are extremely expensive to produce and are not bioavailable due to their susceptibility to proteolytic degradation.

[0099] These issues led to the exploration of alternative protein scaffolds as a source for novel types of protein-based therapeutics. Special attention has been recently given to the use of highly constrained peptides, also known as micro- or mini-proteins, as extremely stable and versatile scaffolds for the production of high affinity ligands for specific protein capture and/or development of therapeutics [28, 29]. Cyclotides are fascinating micro-proteins (~30 residues long) present in plants from the *Violaceae*, *Rubiaceae*, *Cucurbitaceae* and more recently *Fabaceae* families [30]. They display various biological properties such as protease inhibitory, anti-microbial, insecticidal, cytotoxic, anti-HIV and hormone-like

activities [31]. They share a unique head-to-tail circular knotted topology of three disulfide bridges, with one disulfide penetrating through a macrocycle formed by the two other disulfides and inter-connecting peptide backbones, forming what is called a cystine knot topology (**FIG. 2**). Cyclotides belong to the family of knottins, a group of microproteins that also includes conotoxins and spider toxins. Cyclotides can be considered as natural combinatorial peptide libraries structurally constrained by the cystine-knot scaffold and head-to-tail cyclization but in which hypermutation of essentially all residues is permitted with the exception of the strictly conserved cysteines that comprise the knot [32-34]. The main features of cyclotides are a remarkable stability due to the cystine knot, a small size making them readily accessible to chemical synthesis, and an excellent tolerance to sequence variations. For example, the first cyclotide to be discovered, kalata B1, is an orally effective uterotonic [35], and other cyclotides have been shown to cross the cell membrane [36, 37]. More recently, Applicant and other groups have reported engineered cyclotides that target extracellular [38-40] and intracellular [41] targets in animal models. Some of these novel cyclotides are orally bioavailable [39] and are able to cross cellular membranes efficiently [36, 37]. Cyclotides thus appear as highly promising leads or frameworks for peptide drug design [42, 43].

Elimination of specific pairs of BAFF receptors (BR3+BCMA or BR3+TACI) in SLE-mice models

[0100] Although elimination of any single BAFF receptor is incapable of ameliorating development of disease in NZM mice [27], it remained highly plausible that elimination of two BAFF receptors would have a salutary clinical effect. To that end, the Stohl and Jacob laboratories generated NZM mice deficient in two BAFF receptors (NZM.Br3^{-/-}.Bcma^{-/-}, NZM.Br3^{-/-}.Taci^{-/-}, and NZM.Bcma^{-/-}.Taci^{-/-} mice) that, thereby, express only a single BAFF receptor. Surface staining of B cells and plasma cells with mAb specific for the individual BAFF receptors confirmed that the genetically-deleted BAFF receptors were not expressed, whereas the non-deleted BAFF receptor was expressed at normal levels (data not shown).

[0101] In sharp contrast to observations with NZM mice deficient in single BAFF receptors, there are marked divergences in the clinical courses and associated renal immunopathology among the NZM mice doubly-deficient in pairs of BAFF receptors.

NZM.*Bcma*^{-/-}.*Taci*^{-/-} mice develop clinical disease at a more rapid pace than do NZM WT mice (**FIG. 3A**). Whereas >50% of NZM WT mice do not develop severe proteinuria even by 7 months of age, >50% of NZM.*Bcma*^{-/-}.*Taci*^{-/-} mice already develop severe proteinuria by 6 months of age. Moreover, >50% of NZM WT mice are still alive at 10 months of age, whereas >50% of NZM.*Bcma*^{-/-}.*Taci*^{-/-} mice are dead by 7 months of age. This accelerated clinical course is reflected in the renal immunopathology, with glomerular deposition of IgG and C3, glomerular swelling and hypercellularity, and tubular atrophy and interstitial infiltrates all being greater in 6- to 7-month-old NZM.*Bcma*^{-/-}.*Taci*^{-/-} mice than in age-matched NZM WT mice (**FIG. 3B**).

[0102] In contrast, disease onset is significantly delayed and the incidence of clinical disease is significantly reduced in NZM.*Br3*^{-/-}.*Bcma*^{-/-} and NZM.*Br3*^{-/-}.*Taci*^{-/-} mice (**FIG. 3A**). At 12 months of age, >75% of them remain alive and free of clinical disease, whereas almost 90% of NZM WT mice are dead. Renal immunopathology is also greatly attenuated in NZM.*Br3*^{-/-}.*Bcma*^{-/-} and NZM.*Br3*^{-/-}.*Taci*^{-/-} mice, with only modest glomerular deposition of IgG, very little glomerular deposition of C3, and no histologic evidence of glomerular or tubulointerstitial disease at 6-7 months of age when all are prominent in NZM WT mice (**FIG. 3B**).

[0103] Although BCMA and TACI are bound not only by BAFF but by APRIL as well [44, 45], it is highly unlikely that observed efficacy of eliminating BR3 + BCMA or BR3 + TACI is related to loss of APRIL/TACI or APRIL/BCMA interactions, respectively, since NZM mice genetically deficient in APRIL develop features of SLE that are at least as severe as those developed by NZM WT mice [46]. Thus, without being bound by theory, Applicant hypothesizes that agents that either preserve engagement by BAFF with TACI while blocking engagement with BR3 and BCMA or preserve engagement by BAFF with BCMA while blocking engagement with BR3 and TACI may be therapeutically preferable to global inhibition of BAFF.

Cell-based screening of genetically encoded cyclotide-based libraries

[0104] Cyclotides are ribosomally produced in plants from precursors that comprise between one and three cyclotide domains. However, the mechanism of excision of the cyclotide domains and ligation of the free N- and C-termini to produce the circular peptides

has not yet been completely elucidated. Applicant has developed bio-mimetic approaches for the biosynthesis of folded cyclotides inside bacterial cells by making use of modified protein splicing units [47-50]. This important finding opens for the first time the intriguing possibility for the generation of large genetically-encoded libraries of cyclotides (up to $\approx 10^9$) inside living bacterial cells (**FIG. 4**). Genetically-encoded libraries can be readily screened inside the cell using appropriate cell-based reporters for the rapid selection of particular cyclotide sequences that antagonize specific soluble protein targets. In this innovative approach, Applicant can use cell-based libraries (*E. coli* cell libraries) where every single cell expresses a different cyclotide, in what could be labeled a single cell-single compound approach. These compounds are then screened in-cell and selected for their ability to inhibit a particular protein-protein interaction inside the bacterial cell.

[0105] Biologically-generated libraries can be screened inside the cell using appropriate cell-based reporters for the selection of particular cyclotide sequences that bind a specific protein target using high throughput methods. Cell-based screening opens the possibility of using single cells as microfactories where the biosynthesis and screening of particular ligands can take place in a single process within the same cellular cytoplasm [51]. The use of a complex molecular environment, such as the cellular cytoplasm, provides also the ideal background for the selection of highly specific inhibitors. This cell-based screening approach for the selection of particular cyclotide sequences able to inhibit individual BAFF receptors. This is accomplished by using a FRET-based reporter for the in-cell detection of cyclotides able to antagonize BAFF binding to individual receptors (**FIG. 5**). This screening approach is optimized for use in *E. coli* in combination with FACS, and it is designed to screen large libraries while minimizing the number of false positives. This allows easily separating populations of cells in the FRET-ON and FRET-OFF states by FACS (**FIG. 6B**). Applicant also uses a FACS protocol to separate mixtures of cells depending on their FRET-state when using the fluorescence reporter described above. This protocol can be easily used to enrich a population of *E. coli* cells from an initially predominant FRET-ON phenotype (protein complex formed) into a FRET-OFF phenotype (protein complex antagonized). In another model system, an artificial mixture of *E. coli* cells containing cells transfected with pRSF duet plasmids encoding CyPet-HdmX / YPet-Hdm2 (FRET-ON population) and CyPet-Hdm2 / YPet-BARD-I (FRET-OFF population) the population was enriched from a ratio

FRET-OFF:FRET-ON of $1:10^5$ to approximately 100:1 in just 6 rounds of FACS sorting (FIG. 6C).

Cyclotides, Peptides and Polynucleotides

[0106] Cyclotides are small globular microproteins (ranging from 28 to 37 amino acids) with a unique head-to-tail cyclized backbone, which is stabilized by three disulfide bonds forming a cystine-knot motif. This cyclic cystine-knot (CCK) framework provides a rigid molecular platform with exceptional stability towards physical, chemical and biological degradation. These micro-proteins can be considered natural combinatorial peptide libraries structurally constrained by the cystine-knot scaffold and head-to-tail cyclization, but in which hypermutation of essentially all residues is permitted with the exception of the strictly conserved cysteines that comprise the knot. Furthermore, naturally-occurring cyclotides have shown to possess various pharmacologically-relevant activities, and have been reported to cross cell membranes.

[0107] The construction of cyclotides is known in the art and has been described previously (see WO 2011/005598). Synthesis of the peptides and cyclotides useful in the methods and compositions of the disclosure are also described in the Examples that follow.

[0108] Reference herein to a "cyclic backbone" includes a molecule comprising a sequence of amino acid residues or analogues thereof without free amino and carboxy termini. The cyclic backbone of the disclosure comprises sufficient disulfide bonds, or chemical equivalents thereof, to confer a knotted topology on the three-dimensional structure of the cyclic backbone. The term "cyclotide" as used herein refers to a peptide comprising a cyclic cystine knot motif defined by a cyclic backbone, at least two but preferably at least three disulfide bonds and associated beta strands in a particular knotted topology. The knotted topology involves an embedded ring formed by at least two backbone disulfide bonds and their connecting backbone segments being threaded by a third disulfide bond. However, a disulfide bond may be replaced or substituted by another form of bonding such as a covalent bond.

[0109] Thus, in one aspect, the disclosure provides an isolated peptide comprising, or alternatively consisting essentially of, or yet further consisting of a protease recognition leading signal, fused or grafted to cyclotide, optionally comprising a linker and a candidate

BAFF receptor antagonist. In one embodiment, the candidate BAFF receptor antagonist comprises a CDR of an antibody that specifically binds a BAFF receptor. Also provided are first reporter polypeptides comprising a BAFF ligand fused to a reporter element. Further provided are second BAFF receptor polypeptides that bind to the BAFF ligand fused to a reporter element that when in contact with the first reporter polypeptide, emit a detectable signal. In a further embodiment, the linker is inserted at the junctions between the interacting proteins or protein domains and the corresponding fluorescent proteins. Non-limiting examples, peptides for use as a linker comprises, or alternatively consists essentially of, or yet further consists of a peptide selected from the group consisting of [GGS]_n, wherein n is an integer from 1 to 20, GXXGXP, wherein X is any amino acid; GGSGGF; GASGPG; GSGAPG; ASKAPG; or ASRAPG.

[0110] In one aspect, the BAFF receptors are selected from the group of: BR3, TAC1, or BCMA. In one aspect, the BAFF ligand comprises the soluble human BAFF (shBAFF) or a fragment thereof. In another aspect, the fragment comprises the N-terminus of soluble hBAFF, optionally 134-285, and further optionally delta 217-224. In a further aspect, the shBAFF is a mutated shBAFF wherein eight residues at the flap region are replaced by 2 glycine amino acids. In a further aspect, the hBR3 comprises amino acids 1-62. In a yet another embodiment, the hTACI comprises amino acids 68-109. In a yet further aspect, the hBCMA comprises amino acids 1-54. Examples of the amino acid sequence for these proteins are provided herein.

[0111] In a yet further aspect, the BAFF receptor comprises extracellular cysteine-rich CDR BAFF receptor selected from the group of human or murine BR3 (hBR3 or mBR3), human or murine BCMA (hBCMA or mBCMA), or human or murine TACI (hTACI or mTACI), and optionally wherein the receptor is fused to the C-terminus to one of the fluorescent pair, optionally CyPet or YPet.

[0112] In a further aspect, the paired reporters comprises the components of a FRET-based system, optionally a CyPet and a YPet fluorescent protein pair.

[0113] In a further aspect, the flexible linker is inserted at the junctions between the interacting proteins or protein domains and the corresponding fluorescent proteins, and optionally, wherein the linker comprises the amino acid sequence [GGS]_n, wherein n is an

integer from 1 to 20. In another aspect, the linker non-limiting examples of linkers comprise, or alternatively consist essentially of, or yet further consist of a peptide selected from the group consisting of GXXGXP, wherein X is any amino acid; GGSGGF; GASGPG; GSGAPG; ASKAPG; and ASRAPG.

[0114] Unless specifically defined, the peptides can be linear or processed and in their cyclic form.

[0115] In this disclosure, the candidate BAFF receptor antagonist can be grafted into any available loop, e.g., loop 1, or loop 2, or loop 3, or loop 4, or loop 5 or loop 6, of the cyclotide. In one aspect, it is loop 2 or loop 5. The cyclotide comprises a molecular framework comprising a sequence of amino acids forming a cyclic backbone wherein the cyclic backbone comprises sufficient disulfide bonds to confer knotted topology on the molecular framework or part thereof. The cyclic backbone comprises the structure: $C[X^I_1 \dots X_a]C[X^{II}_1 \dots X_b]C[X^{III}_1 \dots X_c]C[X^{IV}_1 \dots X_d]C[X^V_1 \dots X_e]C[X^{VI}_1 \dots X_f]$ wherein C is cysteine; and each of each of $[X^I_1 \dots X_a]$, $[X^{II}_1 \dots X_b]$, $[X^{III}_1 \dots X_c]$, $[X^{IV}_1 \dots X_d]$, $[X^V_1 \dots X_e]$, and $[X^{VI}_1 \dots X_f]$, represents one or more amino acid residues wherein each one or more amino acid residues within or between the sequence residues may be the same or different; and wherein a, b, c, d, e and f represent the number of amino acid residues in each respective sequence and each of a to f may be the same or different and range from 1 to about 20. When the target peptide is grafted into loop 6 of the cyclotide, the amino acid residues corresponding to $[X^{VI}_1 \dots X_f]$ in the cyclotide comprise the target peptide. In some embodiments, the target peptide is grafted into loop 1 of the cyclotide. In this embodiment, the amino acid residues corresponding to $[X^I_1 \dots X_a]$ in the cyclotide comprise the target peptide. In another embodiment, the target peptide is grafted into loop 2 of the cyclotide. In this embodiment, the amino acid residues corresponding to $[X^{II}_1 \dots X_b]$ in the cyclotide comprise the target peptide. In a further embodiment, the target peptide is grafted into loop 3 of the cyclotide. In this embodiment, the amino acid residues corresponding to $[X^{III}_1 \dots X_c]$ in the cyclotide comprise the target peptide. In yet a further embodiment, the target peptide is grafted into loop 4 of the cyclotide. In this embodiment, the amino acid residues corresponding to $[X^{IV}_1 \dots X_d]$ in the cyclotide comprise the target peptide. In another embodiment, the target peptide is grafted into loop 5 of the cyclotide. In this embodiment, the amino acid residues corresponding to $[X^V_1 \dots X_e]$ in the cyclotide comprise the target peptide.

[0116] Additional cyclotide scaffolds or backbones useful in the peptides, methods, and compositions described herein are known in the art and non-limiting examples include, the cyclotides shown in **Table 1**, below.

Table 1

Cyclotide	Protein Sequence
kalata_B1	GLPVCGETCVGGTCNTPGCTCSWPVCTR
cycloviolacin_O1	GIPCAESCVYIPCTVTALLGCSCSNRVCYN
kalata_B2	GLPVCGETCFGGTCNTPGCSTWPICTRD
Palicourein	GDPTFCGETCRVIPVCTYSAALGCTCDDR SDGLCKRN
vhr1	GIPCAESCVWIPCTVTALLGCSCSNKVCY N
tricyclon_A	GGTIFDCGESCFGLGTCYTKGCSCGEWKLC YGTN
circulin_A	GIPCGESCVWIPCISAALGCSCKNKVCYRN
N-KB1-C	GLPVCGETCVGGTCNTPGCTCSWPVCTR
Ac-KB1-C	GLPVCGETCVGGTCNTPGCTCSWPVCTR
N-KB1-Am	GLPVCGETCVGGTCNTPGCTCSWPVCTR
Ac-KB1-Am	GLPVCGETCVGGTCNTPGCTCSWPVCTR
Ac-[desGly]-KB1-Am	LPVCGETCVGGTCNTPGCTCSWPVCTR
kalata_b1-1	TCVGGTCNTPGCTCSWPVCTRNLPCVG
kalata_b1-2	GTCNTPGCTCSWPVCTRNLPCVGETCVG
kalata_b1-3	GCTCSWPVCTRNLPCVGETCVGGTCN
kalata_b1-4	CSWPVCTRNLPCVGETCVGGTCNTPGC
kalata_b1-5	VCTRNLPCVGETCVGGTCNTPGCTCS
kalata_b1-6a	VCGETCVGGTCNTPGCTCSWPVCT
kalata_b1-6b	RNGLPCVGETCVGGTCNTPGCTCSWPVCT
cycloviolacin_O2	GIPCGESCVWIPCISSAIGCSCKSKVCYRN
des(24-28)kB1	VCGETCVGGTCNTPGCTCSWPVCT

Cylcotide	Protein Sequence
[Ala1,15]kB1	GLPVAGETCVGGTCNTPGATCSWPVCTR N
kalata_B6	GLPTCGETCFGGTCNTPGCSCSSWPICTRN
kalata_B3	GLPTCGETCFGGTCNTPGCTCDPWPICTR D
kalata_B7	GLPVCGETCTLGTCYTQGCTCSWPICKRN
cycloviolacin_O8	GTLPCGESCVWIPCISAVVGCSCSKSKVCYK N
cycloviolacin_O11	GTLPCGESCVWIPCISAVVGCSCSKSKVCY KN
kalata_B4	GLPVCGETCVGGTCNTPGCTCSWPVCTR D
vodo_M	GAPICGESCVFTGKCYTVQCSCSWPVCTR N
cyclopsychotride_A	SIPCGESCVFIPCTVTALLGCSCSKSKVCYK N
cycloviolacin_H1	GIPCGESCVYIPCLTSAIGCSCKSKVCYRN
cycloviolacin_O9	GIPCGESCVWIPCLTSAVGCSCSKSKVCYR N
vico_A	GSIPCAESCVYIPCFITGIAGCSCKNKVCYY N
vitri_A	GIPCGESCVWIPCITSAIGCSCKSKVCYRN
kalata_S	GLPVCGETCVGGTCNTPGCSCSWPVCTR N
cycloviolacin_O12	GLPICGETCVGGTCNTPGCSCSWPVCTR N
vodo_N	GLPVCGETCTLGKCYTAGCSCSWPVCYR N
vico_B	GSIPCAESCVYIPCITGIAGCSCKNKVCYY N
kalata_B1_Ila	GLPVCGETCVGGTCNTPGCTCSWPVCTR N
Hypa_A	GIPCAESCVYIPCTITALLGCSCCKNKVCY N

Cylcotide	Protein Sequence
circulin_B	GVIPCGESCVFIPCISTLLGCSCKNKVCYR N
circulin_C	GIPCGESCVFIPCITSVAGCCKSKVCYRN
circulin_D	KIPCGESCVWIPCITSIFNCKCENKVCYHD
circulin_E	KIPCGESCVWIPCLTSVFNCKCENKVCYH D
circulin_F	AIPCGESCVWIPCISAAIGCSCKNKVCYR
cycloviolacin_O4	GIPCGESCVWIPCISSAIGCSCKNKVCYRN
cycloviolacin_O3	GIPCGESCVWIPCLTSAIGCCKSKVCYRN
cycloviolacin_O5	GTPCGESCVWIPCISSAVGCSCKNKVCYK N
cycloviolacin_O6	GTLPCGESCVWIPCISAAVGCCKSKVCY KN
cycloviolacin_O7	SIPCGESCVWIPCTITALAGCKCKSKVCYN
cycloviolacin_O10	GIPCGESCVYIPCLTSAVGCCKSKVCYRN
kalata_B5	GTPCGESCVYIPCISGVIGCSCTDKVCYLN
varv_peptide_B	GLPVCGETCFGGTCNTPGCSCDPWPMCSR N
varv_peptide_C	GVPICGETCVGGTCNTPGCSCSWPVCTR
varv_peptide_D	GLPICGETCVGGSCNTPGCSCSWPVCTR
varv_peptide_F	GVPICGETCTLGTCYTAGCSCSWPVCTR
varv_peptide_G	GVPVCGETCFGGTCNTPGCSCDPWPVCSR N
varv_peptide_H	GLPVCGETCFGGTCNTPGCSCETWPVCSR N
cycloviolacin_A	GVIPCGESCVFIPCISAAIGCSCKNKVCYRN
cycloviolacin_B	GTACGESCVLPCFTVGCTCTSSQCFKN
cycloviolacin_C	GIPCGESCVFIPCLTTVAGCCKSKVCYRN
cycloviolacin_D	GFPCGESCVFIPCISAAIGCSCKNKVCYRN

Cylcotide	Protein Sequence
violapeptide_1	GLPVCGETCVGGTCNTPGCSCSRPVCTXN
vhl-1	SISCGESCAMISFCFTEVIGCSCKNKVCYL N
Vontr_Protein	ALETQKPNHLEEALVAFAKKGNLGGLP
hcf-1	GIPCGESCHYIPCVTSAIGCSCRNRSCMRN
htf-1	GIPCGDSCHYIPCVTSTIGCSCTNGSCMRN
Oantr_protein	GVKSSSETTLMFLKEMQLKLP
vhl-2	GLPVCGETCFTGTCTNGCTCDPWPVCTR N
cycloviolacin_H3	GLPVCGETCFGGTCNTPGCICDPWPVCTR N
cycloviolacin_H2	SAIACGESCVYIPCFIPGCSCRNRVCYLN
Hyfl_A	SISCGESCVYIPCTVTALVGCTCKDKVCYL N
Hyfl_B	GSPIQCAETCFIGKCYTEELGCTCTAFLCM KN
Hyfl_C	GSPRQCAETCFIGKCYTEELGCTCTAFLC MKN
Hyfl_D	GSVPCGESCVYIPCFTGIAGCSCKSKVCYY N
Hyfl_E	GEIPCGESCVYLPCLPNCYCRNHVCYLN
Hyfl_F	SISCGECTTFNCWIPNCKCNHHDKVCYW N
Hyfl_G_(partial)	CAETCVVLPCFIVPGCSCKSSVCYFN
Hyfl_H_(partial)	CAETCIYIPCFTEAVGCKCKDKVCYKN
Hyfl_I	GIPCGESCVFIPCISGVIGCSCKSKVCYRN
Hyfl_J	GIACGESCA YFGCWIPGCSCRNKVCYFN
Hyfl_K	GTPCGESCVYIPCFTAVVGCTCKDKVCYL N

Cylcotide	Protein Sequence
Hyfl_L	GTPCAESCVYLPCFTGVIGCTCKDKVCYL N
Hyfl_M	GNIPCGESCIFPCFNPGCSCCKDNLCCYYN
Hyfl_N_(partial)	CGETCVILPCISAALGCSCCKDTVCCYKN
Hyfl_O_(partial)	CGETCVIFPCISAAFGCSCCKDTVCCYKN
Hyfl_P	GSVPCGESCVWIPCISGIAGCSCCKNKVCYL N
Hymo_A_(partial)	CGETCLFIPCIFSVVGCSCSSKVCYRN
Hymo_B_(partial)	CGETCVTGTCYTPGCACDWPVCKRD
Hyst_A_(partial)	CGETCIWGRCYSENIGCHCGFGICTLN
Hyve_A_(partial)	CGETCLFIPCLTSVFGCSCCKNRGCYKI
HycA_A_(partial)	CGETCVVDTRCYTKKCSAWPVCMRN
Hyde_A_(partial)	CVWIPCISAAIGCSCCKSKVCYRN
Hyen_A_(partial)	CGESCVYIPCTVTALLGCSCCKDKVCYKN
Hyen_B_(partial)	CGETCKVTKRCSGQGCSCCLKGRSCYD
Hyep_A_(partial)	CGETCVVLPCFIVPGCSCCKSSVCYFN
Hyep_B_(partial)	CGETCIYIPCFTEAVGCKCKDKVCYKN
tricyclon_B	GGTIFDCGESCFLGTCYTKGCSCGEWKLC YGEN
kalata_B8	GSVLNCGETCLLGTCYTTGCTCNKYRVCT KD
cycloviolacin_H4	GIPCAESCVWIPCTVTALLGCSCSNNVCY N
cycloviolacin_O13	GIPCGESCVWIPCISAAIGCSCCKSKVCYRN
violacin_A	SAISCGETCFKFKCYTPRCSCSYPVCK
cycloviolacin_O14	GSIPACGESCFKGKCYTPGCSCSKYPLCAK N
cycloviolacin_O15	GLVPCGETCFTGKCYTPGCSCSYPICKKN
cycloviolacin_O16	GLPCGETCFTGKCYTPGCSCSYPICKKIN

Cylcotide	Protein Sequence
cycloviolacin_O17	GIPCGESCVWIPCISAAIGCSCKNKVCYRN
cycloviolacin_O18	GIPCGESCVYIPCTVTALAGCKCKSKVCY N
cycloviolacin_O19	GTLPCGESCVWIPCISSVVGCSCKSKVCYK D
cycloviolacin_O20	GIPCGESCVWIPCLTSAIGCSCKSKVCYRD
cycloviolacin_O21	GLPVCGETCVTGSCYTPGCTCSWPVCTR
cycloviolacin_O22	GLPICGETCVGGTCNTPGCTCSWPVCTR
cycloviolacin_O23	GLPTCGETCFGGTCNTPGCTCDSSWPICTH N
cycloviolacin_O24	GLPTCGETCFGGTCNTPGCTCDPWPVCTH N
cycloviolacin_O25	DIFCGETCAFIPCITHVPGTCSCSKSKVCYFN
[P20D,V21K]- kalata_B1	GLPVCGETCVGGTCNTPGCTCSWDKCTR N
[W19K,_P20N,_V21K]-kalata_B1	GLPVCGETCVGGTCNTPGCTCSKNKCTR
[Glu(Me)]cyO2	GIPCGXSCVWIPCISSAIGCSCKSKVCYRN
[Lys(Ac)]2cyO2	GIPCGESCVWIPCISSAIGCSCXSXVCYRN
[Arg(CHD)]cyO2	GIPCGESCVWIPCISSAIGCSCKSKVCYXN
([Lys(Ac)]2[Arg(CHD)])cyO2	GIPCGESCVWIPCISSAIGCSCXSXVCYXN
kalata_B1_oia	GLPVCGETCVGGTCNTPGCTCSWPVCTR
kalata_B1_nfk	GLPVCGETCVGGTCNTPGCTCSWPVCTR
kalata_B2_nfk	GLPVCGETCFGGTCNTPGCSTWPICTRD
kalata_B2_kyn	GLPVCGETCFGGTCNTPGCSTWPICTRD
kalata_B9	GSVFNCGETCVLGTCYTPGCTCNTYRVCT KD
kalata_B10	GLPTCGETCFGGTCNTPGCSCSSWPICTRD

Cylcotide	Protein Sequence
kalata_B10_oia	GLPTCGETCFGGTCNTPGCSCSSWPICTRD
kalata_B11	GLPVCGETCFGGTCNTPGCSCDPICTRD
kalata_B12	GSLCGDTCFVLGCNDSSCSCNYPICVKD
kalata_B13	GLPVCGETCFGGTCNTPGCACDPWPVCTR D
kalata_B14	GLPVCGESCFGGTCNTPGCACDPWPVCTR D
kalata_B15	GLPVCGESCFGGSCYTPGCSCDWPICTRD
kalata_B16	GIPCAESCVYIPCTITALLGCKCQDKVCYD
kalata_B17	GIPCAESCVYIPCTITALLGCKCKDQVCYN
Amrad_5	CGETCVGGTCNTPGCTCSWPVCRRKRRR
Amrad_9	CGETCRRKRRRCNTPGCTCSWPVCTRNL PV
Amrad_11	CGETCVGGTCNTRRKRRRGCTCSWPVCT RNLGPV
Amrad_17	CGETCVGGTCNTPGCTCRRKRRRVCTRNL GLPV
Amrad_7	CGETCVGGTCNTPGCTCRRKRRRCNTRL PV
Amrad_8	CGETCVGGTCRRKRRRCTCSWPVCTRNL LPV
kalata_B18	GVPCAESCVYIPCISTVLGCSCSNQVCYRN
PS-1	GFIPCGETCIWDKTCHAAGCSCSVANICVR N
CD-1	GADGFCGESCVYIPCISYLVGCSCDTIEKV CKRN
cycloviolacin_Y1	GGTIFDCGETCFLGTCYTPGCSCGNYGFC YGTN

Cylcotide	Protein Sequence
cycloviolacin_Y2	GGTIFDCGESCF LGTCYTAGCSCGNWGLC YGTN
cycloviolacin_Y3	GGTIFDCGETCFLGTCYTAGCSCGNWGLC YGTN
cycloviolacin_Y4	GVPCGESCVFIPCITGVIGCSCSSNVCYLN
cycloviolacin_Y5	GIPCAESCVWIPCTVTALVGCSCSDKVCY N
vibi_A	GLPVCGETCFGGTCNTPGCSCSYPICTR N
vibi_B	GLPVCGETCFGGTCNTPGCTCSYPICTR N
vibi_C	GLPVCGETCAFGSCYTPGCSCSWPVCTR N
vibi_D	GLPVCGETCFGGRCNTPGCTCSYPICTR N
vibi_E	GIPCAESCVWIPCTVTALIGCGCSNKVCYN
vibi_F	GTIPCGESCVFIPCLTSA LGCSCKSKVCYK N
vibi_G	GTFPCGESCVFIPCLTSAIGCSCKSKVCYK N
vibi_H	GLLPCAESCVYIPCLTTVIGCSCKSKVCYK N
vibi_I	GIPCGESCVWIPCLTSTVGCSCSKSKVCYRN
vibi_J	GTFPCGESCVWIPCISKVIGCACKSKVCYK N
vibi_K	GIPCGESCVWIPCLTSAVGCPCSKSKVCYR N
Viba_2	GIPCGESCVYLP CFTAPLGCS CSSKVCYRN
Viba_5	GIPCGESCVWIPCLTATIGCSCKSKVCYRN
Viba_10	GIPCAESCVYLP CVTIVIGCSCKDKVCYN
Viba_12	GIPCAESCVWIPCTVTALLGCSCCKDKVCY N

Cylcotide	Protein Sequence
Viba_14	GRLCGERCVIERTRAWCRTVGCICSLHTL ECVRN
Viba_17	GLPVCGETCVGGTCNTPGCGCSWPVCTR N
Viba_15	GLPVCGETCVGGTCNTPGCACSWPVCTR N
mram_1	GSIPCGESCVYIPCISSLLGCSCSKSKVCYKN
mram_2	GIPCAESCVYIPCLTSAIGCSCKSKVCYRN
mram_3	GIPCGESCVYLPCTTTIIGCKCQGKVCYH
mram_4	GSIPCGESCVFIPCISSVVGCSCKNKKVCYK N
mram_5	GTIPCGESCVFIPCLTSAIGCSCKSKVCYKN
mram_6	GSIPCGESCVYIPCISSLLGCSCSKVCYKN
mram_7	GSIPCGESCVFIPCISSIVGCSCSKSKVCYKN
mram_8	GIPCGESCVFIPCLTSAIGCSCKSKVCYRN
mram_9	GVPCGESCVWIPCLTSIVGCSCCKNNVCTL N
mram_10	GVIPCGESCVFIPCISSVLGCSCCKNKKVCYR N
mram_11	GHPTCGETCLLGTCYTPGCTCKRPVCYKN
mram_12	GSAILCGESCTLGECYTPGCTCSWPICKN
mram_13	GHPICGETCVGNKCYTPGCTCTWPVCYRN
mram_14	GSIPCGEGCVFIPCISSIVGCSCSKSKVCYKN
Viba_1	GIPCGEGCVYLPCTAPLGCSCSSKVCYRN
Viba_3	GIPCGESCVWIPCLTAAIGCSCSSKVCYRN
Viba_4	GVPCGESCVWIPCLTSAIGCSCKSSVCYRN
Viba_6	GIPCGESCVLIPCISSVIGCSCKSKVCYRN
Viba_7	GVIPCGESCVFIPCISSVIGCSCKSKVCYRN
Viba_8	GAGCIETCYTFPCISEMINCSCKNSRCQKN

Cylcotide	Protein Sequence
Viba_9	GIPCGESCVWIPCISSAIGCSCKNKVCYRK
Viba_11	GIPCGESCVWIPCISGAIGCSCKSKVCYRN
Viba_13	TIPCAESCVWIPCTVTALLGCSCDKVCY N
Viba_16	GLPICGETCTLGTCYTVGCTCSWPICTRN
[G1A]kalata_B1	ALPVCGETCVGGTCNTPGCTCSWPVCTR
[L2A]kalata_B1	GAPVCGETCVGGTCNTPGCTCSWPVCTR N
[P3A]kalata_B1	GLAVCGETCVGGTCNTPGCTCSWPVCTR N
[V4A]kalata_B1	GLPACGETCVGGTCNTPGCTCSWPVCTR
[G6A]kalata_B1	GLPVCAETCVGGTCNTPGCTCSWPVCTR
[E7A]kalata_B1	GLPVCGATCVGGTCNTPGCTCSWPVCTR N
[T8A]kalata_B1	GLPVCGEACVGGTCNTPGCTCSWPVCTR N
[V10A]kalata_B1	GLPVCGETCAGGTCNTPGCTCSWPVCTR
[G11A]kalata_B1	GLPVCGETCVAGTCNTPGCTCSWPVCTR
[G12A]kalata_B1	GLPVCGETCVGATCNTPGCTCSWPVCTR
[T13A]kalata_B1	GLPVCGETCVGGACNTPGCTCSWPVCTR N
[N15A]kalata_B1	GLPVCGETCVGGTCATPGCTCSWPVCTR
[T16A]kalata_B1	GLPVCGETCVGGTCNAPGCTCSWPVCTR N
[P17A]kalata_B1	GLPVCGETCVGGTCNTAGCTCSWPVCTR N
[G18A]kalata_B1	GLPVCGETCVGGTCNTPACTCSWPVCTR
[T20A]kalata_B1	GLPVCGETCVGGTCNTPGCACSWPVCTR N

Cylcotide	Protein Sequence
[S22A]kalata_B1	GLPVCGETCVGGTCNTPGCTCAWPVCTR N
[W23A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSAPVCTR N
[P24A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSWAVCTR N
[V25A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSWPACTRN
[T27A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSWPVCAR N
[R28A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSWPVCTA N
[N29A]kalata_B1	GLPVCGETCVGGTCNTPGCTCSWPVCTRA
Cter_A	GVIPCGESCVFIPCISTVIGCSCKNKVCYRN
Cter_B	GVPCAESCVWIPCTVTALLGCSCCKDKVCY LN
hcf-1_variant	GIPCGESCHIPCVTSAIGCSCRNRSCMRN
Vpl-1	GSQSCGESCVLIPCISGVIGCSCSSMICYFN
Vpf-1	GIPCGESCVFIPCLTAAIGCSCRSKVCYRN
cO31	GLPVCGETCVGGTCNTPGCSCSIPVCTR N
cO28	GLPVCGETCVGGTCNTPGCSCSWPVCFRD
cO32	GAPVCGETCFGGTCNTPGCTCDPWPVCTN D
cO33	GLPVCGETCVGGTCNTPYCTCSWPVCTR D
cO34	GLPVCGETCVGGTCNTEYCTCSWPVCTR D
cO35	GLPVCGETCVGGTCNTPYCFCSWPVCTR D
cO29	GIPCGESCVWIPCISGAIGCSCSKSKVCYKN
cO30	GIPCGESCVWIPCISSAIGCSCKNKVCFKN
cO26	GSIPACGESCFRGKCYTPGCSCSKYPLCAK D

Cylcotide	Protein Sequence
cO27	GSIPACGESCFKGWCYTPGCSCSKYPLCA KD
Globa_F	GSFPCGESCVFIPCISAIAGCSCKNKVCYK N
Globa_A	GIPCGESCVFIPCITAAIGCSCKTKVCYRN
Globa_B	GVIPCGESCVFIPCISAVLGCSCSKSKVCYR N
Globa_D	GIPCGETCVFMPCISGPMGCSCCKHMCYR N
Globa_G	GVIPCGESCVFIPCISSVLGCSCCKNKVCYR N
Globa_E	GSAFGCGETCVKGKCNTPGCVCSWPVCK KN
Globa_C	APCGESCVFIPCISAVLGCSCSKSKVCYRN
Glopa_D	GVPCGESCVWVPCTVTALMGCSVCREVC RKD
Glopa_E	GIPCAESCVWIPCTVTKMLGCSCCKDKVCY N
Glopa_A	GGSIPIETCVWTGCFLVPGCSCKSDKKCY LN
Glopa_B	GGSVPIETCVWTGCFLVPGCSCKSDKKC YLN
Glopa_C	GDIPLCGETCFEGGNCRIPGCTCVWPFCSK N
Co36	GLPTCGETCFGGTCNTPGCTCDPFPVCTH D
cycloviolacin_T1	GIPVCGETCVGGTCNTPGCSCSWPVCTR
cycloviolacin_T2	GLPICGETCVGGTCNTPGCSCSWPVCTR
psyle_A	GIACGESCVFLGCFIPGCSCSKSKVCYFN

Cyclotide	Protein Sequence
psyle_B	GIPCGETCVAFGCWIPGCSCDKKLCYYD
psyle_C	KLCGETCFKFKCYTPGCSCSYFPCK
psyle_D	GIPCGESCVFIPCTVTALLGCSCQNKVCYR D
psyle_E	GVIPCGESCVFIPCISSVLGCSCKNKVCYR D
psyle_F	GVIPCGESCVFIPCITAAVGCSCKNKVCYR D
vaby_A	GLPVCGETCAGGTCNTPGCSCSWPICTRN
vaby_B	GLPVCGETCAGGTCNTPGCSCSWPICTRN
vaby_C	GLPVCGETCAGGRCNTPGCSCSWPVCTR
vaby_D	GLPVCGETCFGGTCNTPGCTCDPWPVCTR N
vaby_E	GLPVCGETCFGGTCNTPGCSCDPWPVCTR N
Oak6_cyclotide_2	GLPICGETCFGGTCNTPGCICDPWPVCTR
Oak7_cyclotide	GSHCGETCFFFGCYKPGCSCDELRCYKN
Oak8_cyclotide	GVPCGESCVFIPCLTAVVGCSCSNKVCYL N
Oak6_cyclotide_1	GLPVCGETCFGGTCNTPGCACDPWPVCTR N
Cter_C	GVPCAESCVWIPCTVTALLGCSCDKKVCY LD
Cter_D	GIPCAESCVWIPCTVTALLGCSCDKKVCY LN
Cter_E	GIPCAESCVWIPCTVTALLGCSCDKKVCY LD
Cter_F	GIPCGESCVFIPCISSVVGCSCKSKVCYLD
Cter_G	GLPCGESCVFIPCITTVVGCSCKNKVCYNN

Cyclotide	Protein Sequence
Cter_H	GLPCGESCVFIPCITTVVGCSCKNKVCYND
Cter_I	GTVPCGESCVFIPCITGIAGCSCKNKVCYIN
Cter_J	GTVPCGESCVFIPCITGIAGCSCKNKVCYID
Cter_K	HEPCGESCVFIPCITTVVGCSCKNKVCYN
Cter_L	HEPCGESCVFIPCITTVVGCSCKNKVCYD
Cter_M	GLPTCGETCTLGTCYVPDCSCSWPICMKN
Cter_N	GSAFCGETCVLGTCYTPDCSCTALVCLKN
Cter_O	GIPCGESCVFIPCITGIAGCSCKSKVCYRN
Cter_P	GIPCGESCVFIPCITAAIGCSCKSKVCYRN
Cter_Q	GIPCGESCVFIPCISTVIGCSCKNKVCYRN
Cter_R	GIPCGESCVFIPCTVTALLGCCKDKVCYK N
vitri_B	GVPICGESCVGGTCNTPGCSCSWPVCTTN
vitri_C	GLPICGETCVGGTCNTPGCFCTWPVCTRN
vitri_D	GLPVCGETCFTGSCYTPGCSCNWPVCNRN
vitri_E	GLPVCGETCVGGTCNTPGCSCSWPVCFRN
vitri_F	GLTPCGESCVWIPCISSVGCACKSKVCY KD
hedyotide_B1	GTRCGETCFVLPCWSAKFGCYCQKGFCY RN

[0117] In another aspect, this disclosure provides an isolated polynucleotide encoding one or more of the isolated cyclotide peptides described above, alone or in a gene delivery vehicle (e.g., liposome, biocompatible polymers, lipoproteins, artificial viral envelopes, plasmids, viral vectors) a replication or an expression vector, e.g., a viral vector or a plasmid. Also provided are isolated complementary sequences to the isolated polynucleotide encoding one or more of the isolated peptides described above. The polynucleotides can further contain the necessary regulatory element(s) operatively linked to the coding sequences for expression of

the polynucleotide in a host cell. In a further aspect, the isolated polynucleotides can further comprise a detectable label, e.g. a radioactive or fluorescent label.

[0118] In one aspect, provided herein is a first expression vectors encoding a cyclotide comprising the candidate BAFF receptor antagonist, and wherein the first expression vectors further comprise a first origin of replication. Also provided is a reporter plasmid encoding a BAFF ligand and a plasmid encoding one or more BAFF receptors, wherein each reporter plasmid is distinct from the first expression vector and comprises a distinct origin of replication from the first origin of replication. In one aspect, the polynucleotide encoding the candidate BAFF receptor antagonist encodes a CDR of an antibody that specifically binds a BAFF receptor. Examples of BAFF receptors are selected from the group of: BR3, TAC1, or BCMA. In a further aspect, the candidate receptor antagonist specifically binds the receptors BR3 and TAC1, but not BCMA. In one aspect, the plasmid encodes a BAFF candidate receptor antagonist specifically binds the receptors BR3 and BCMA, but not TAC1. In one aspect, the linker is a flexible linker and the polynucleotide encoding such is inserted in the plasmid at the junctions between the polynucleotides encoding the interacting proteins or protein domains. Non-limiting examples, peptides for use as a linker comprises, or alternatively consists essentially of, or yet further consists of a peptide selected from the group consisting of GXXGXP, wherein X is any amino acid; GGSGGF; GASGPG; GSGAPG; ASKAPG; and ASRAPG. In one aspect the first expression vector is a pASK expression vector and/or the expression vector of the reporter plasmid comprises pRSF-det and/or pBAD33. As is apparent to the skilled artisan, vectors other than plasmids can be substituted for plasmids and are selected for use in the selected host cell system, e.g., prokaryotic, eukaryotic, E. coli, mammalian cell, or for example, a yeast cell.

[0119] In a further aspect, the plasmid encoding the BAFF ligand comprises a polynucleotide encoding the soluble human BAFF (shBAFF) or a fragment thereof. In one embodiment, the polynucleotide encodes a fragment that comprises the N-terminus of soluble hBAFF, optionally 134-285, and further optionally delta 217-224. In a further aspect, the polynucleotide encodes a shBAFF that is a mutated shBAFF wherein eight residues at the flap region are replaced by 2 glycine amino acids. In a yet further aspect, the at least one reporter plasmid encodes an extracellular cysteine-rich CDR BAFF receptor selected from the group of human or murine BR3 (hBR3 or mBR3), human or murine BCMA (hBCMA or

mBCMA), or human or murine TACI (hTACI or mTACI). In a yet further aspect, hBR3 comprises amino acids 1-62. In a yet further aspect, the hTACI comprises amino acids 68-109. In another aspect, the hBCMA comprises amino acids 1-54.

[0120] The isolated peptides and polynucleotides of this invention can be produced by chemical synthetic methods or by recombinant expression of isolated polynucleotides as described herein or using methods known to those of skill in the art.

Host Cells and Compositions

[0121] This disclosure also provides an isolated host cell comprising one or more of: the peptides as described above, an isolated polynucleotide, a gene delivery vehicle or vector containing the isolated polynucleotides. The isolated host cell is a prokaryotic or a eukaryotic cell. In one particular aspect, the host cell is an E. coli cell.

[0122] “Host cell” refers not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[0123] Examples of such include, prokaryotic cells such as E. coli cells. Examples of eukaryotic cells include, but are not limited to cells from animals, e.g., murines, rats, rabbit, simians, bovines, ovine, porcine, canines, feline, farm animals, sport animals, pets, equine, and primate, particularly human. The cells can be cultured cells or they can be primary cells. Cultured cell lines can be purchased from vendors such as the American Type Culture Collection (ATCC), U.S.A.

[0124] The host cells can be used for recombinant production of the polynucleotides or peptides of this invention or to provide a screen for therapeutic agents. Further provided is a method for recombinantly producing the peptides of this disclosure by growing an isolated host cell as described above under conditions that favor the expression of the polynucleotide. In one aspect, the peptides are isolated from the host cells.

[0125] Any of the above noted compositions can be combined with a carrier, such as a pharmaceutically acceptable carrier.

Methods and Uses

[0126] This disclosure also provides methods for recombinantly producing peptides and polynucleotides of this invention by culturing a host cell the isolated polynucleotides, the plasmid or vector containing the polynucleotide encoding the peptide, under conditions for the expression of the polynucleotide to peptide. In a further aspect, the peptide is isolated from the cell.

[0127] As is apparent to one of skill in the art, suitable cells may be cultured in microtiter plates and several agents may be assayed at the same time by noting genotypic changes, phenotypic changes and/or cell death.

[0128] In one aspect, provided herein is a method for isolating a candidate BAFF receptor antagonist, comprising:

- a) inserting into a cell one or more first expression vectors encoding a cyclotide comprising the candidate BAFF receptor antagonist, and wherein the first expression vectors further comprise a first origin of replication;
- b) inserting into the cell a reporter expression vector, optionally a plasmid encoding a BAFF ligand and an expression vector, optionally a plasmid encoding one or more BAFF receptors, wherein each reporter plasmid is distinct from the first expression vector and comprises a distinct origin of replication from the first origin of replication;
- c) inducing expression of the candidate antagonist;
- d) inducing expression of the BAFF ligand and the one or more receptors; and
- e) selecting cells wherein the candidate BAFF receptor antagonist binds to the BAFF receptor, thereby isolating the candidate BAFF receptor antagonist.

[0129] In one aspect, the cells of steps a) and/or b) are prokaryotic or eukaryotic, optionally *E. coli* cells.

[0130] In one embodiment, the reporter of step b) comprises the components of a FRET-based system, optionally a CyPet and a YPet fluorescent protein pair, one member on the receptor and one member on the ligand.

[0131] In a further aspect, a linker such as a flexible linker is inserted at the junctions between the interacting proteins or protein domains and the corresponding fluorescent proteins, and optionally, wherein the linker comprises the amino acid sequence [GGG]_n, wherein n is an integer from 1 to 20.

[0132] Any appropriate expression vector can be used. A non-limiting example of such comprises the expression vector of step a) being a pASK expression vector and/or the expression vector of the reporter of step b) comprises pRSF-det and pBAD33.

[0133] In one aspect, the candidate BAFF receptor antagonist encodes CDR of an antibody that specifically binds a BAFF receptor. In one aspect, step b) is performed before step a).

[0134] In a further aspect, the one or more BAFF receptors are selected from the group of: BR3, TAC1, or BCMA. In cell of step b) comprises a vector encoding a BR3 receptor and a vector encoding a TAC1 receptor. In a further aspect, the cell of step b) comprises a vector encoding a BR3 receptor and a BCMA receptor. In another aspect, the cell of step b) comprises one or more vectors encoding a BR3 receptor, a TAC1 receptor or a BCMA receptor. In one embodiment, the method selects for a candidate receptor antagonist specifically binds the receptors BR3 and TACI, but not BCMA. In another aspect, the method selects for a candidate receptor antagonist specifically binds the receptors BR3 and BCMA, but not TACI. In a further aspect, the BAFF ligand comprises the soluble human BAFF (shBAFF) or a fragment thereof. In another aspect, the fragment comprises the N-terminus of soluble hBAFF, optionally 134-285, and further optionally delta 217-224. In another aspect, the shBAFF is a mutated shBAFF wherein eight residues at the flap region are replaced by 2 glycine amino acids. In another aspect, at least one reporter vector encodes an extracellular cysteine-rich CDR BAFF receptor selected from the group of human or murine BR3 (hBR3 or mBR3), human or murine BCMA (hBCMA or mBCMA), or human or murine TACI (hTACI or mTACI), and optionally wherein the receptor is fused to the C-terminus to one of the fluorescent pair, optionally CyPet or YPet.

[0135] In one aspect of the method the hBR3 comprises amino acids 1-62. In another aspect of the method, the hTACI comprises amino acids 68-109. In a yet further aspect of the method, the hBCMA comprises amino acids 1-54.

[0136] In a further aspect, the method further comprises isolating BAFF receptor antagonist of step e) and further inserting the isolated candidate receptor antagonist into a second or further system comprising a vector encoding a BAFF receptor that is different from the BAFF receptor of step b) and repeating steps c) through e).

[0137] The methods can be performed in a cell or the proteins can be brought in contact with each other in a test tube, in vitro. As exemplified in **FIG. 5**, candidate receptor antagonist that prohibit the binding of the ligand and receptor will prohibit the recognition and binding of the elements of the reporter pair.

[0138] In a further aspect, the candidate BAFF receptor antagonist that has the preferred binding affinity is isolated and further characterized by repeating the method with different ligand/receptor pairs or other possible binding partners. The antagonist can be further characterized and sequenced. In one embodiment, the candidate is characterized by a method comprising mapping the molecular interaction, optionally by NMR.

[0139] The candidate receptor antagonists are isolated and further assayed for additional in vitro activity and isolating the candidate with the in vitro activity.

[0140] With appropriate validation, the receptor antagonist is used in a for inhibiting the interaction of BAFF with a BAFF receptor comprising contacting a cell or solution comprising the BAFF and the BAFF receptor with the receptor antagonist. The contacting can be in vitro, ex vivo or in vivo.

[0141] Also provided is a method for treating SLE comprising administering to a subject suffering from SLE an effective amount of the BAFF receptor antagonist.

[0142] Further provided is a method for treating a disease or condition that can be treated by inhibiting the binding of a BAFF ligand to a BAFF receptor, comprising, or alternatively consisting essentially of, or yet further consisting of, administering to a subject in need of such treatment an effective amount of one or more of the receptor antagonist.

[0143] The compositions can be administered to an animal or mammal by a treating veterinarian or to a human patient by a treating physician.

[0144] The antagonist is administered in an effective amount to treat the condition or disease and by any suitable means and with any suitable formulation as a composition and

therefore includes a carrier such as a pharmaceutically acceptable carrier. Accordingly, a formulation comprising the necessary antagonist is further provided herein. The formulation can further comprise one or more preservatives or stabilizers. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (e.g., 0.005, 0.01), 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g., 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, and 1.0%). The term carrier can include a buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Representative buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. Additional carriers include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrans (e.g., cyclodextrins, such as 2-hydroxypropyl- β -cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g., polysorbates such as “TWEEN 20” and “TWEEN 80”), lipids (e.g., phospholipids, fatty acids), steroids (e.g., cholesterol), and chelating agents (e.g., EDTA).

[0145] An “effective amount” is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages.

[0146] The invention provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of the antagonist as described herein and/or preservatives, optionally in an aqueous diluent, wherein said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. The invention further comprises an article of manufacture, comprising packaging material, a first vial comprising the antagonist

and/or a second vial comprising an aqueous diluent of prescribed buffer or preservative, wherein said packaging material comprises a label that instructs a physician, technician or patient to reconstitute the therapeutic in the aqueous diluent to form a solution that can be held over a period of twenty-four hours or greater.

[0147] Various delivery systems are known and can be used to administer an antagonist of the disclosure, e.g., direct delivery, encapsulation in liposomes, microparticles, microcapsules. Methods of delivery include but are not limited to intra-arterial, intra-muscular, intravenous, intranasal and oral routes. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, by injection or by means of a catheter.

Kits

[0148] In a yet further aspect, a kit is provided that comprises, or alternatively consists essentially of, or yet further consisting of, any of one or more of the isolated polypeptide as disclosed above, an isolated polynucleotide as disclosed herein, an isolated vector or host cell as described above, or the compositions, and instructions for use.

[0149] Having described the general concepts of this invention, the following illustrative examples are provided.

EXPERIMENTAL DESIGN AND METHODS

Biosynthesis of genetically-encoded libraries of cyclotides

[0150] Cyclotide-based libraries are created at the DNA level using double stranded DNA inserts with degenerate sequences for some of the different loops of the cyclotide scaffold (i.e. loops 1, 2, 3 and/or 5, see **FIG. 2**). Briefly, a long degenerate synthetic oligonucleotide (which encodes the whole cyclotide, ≈ 100 nucleotide-long) template is PCR amplified using 5'- and 3'-primers corresponding to the non-degenerate flanking regions. The resulting double-stranded degenerate DNA is double digested and then ligated to a linearized protein splicing unit (intein)-encoding expression vector to produce a library of plasmids. These libraries are then transformed into electrocompetent *E. coli* cells to finally obtain a library of cells containing typically up to $\approx 10^9$ different clones (i.e. cyclotide sequences). The

degenerate synthetic oligonucleotide template is synthesized using a NN(G/T) codon scheme for the randomized loops. This scheme uses 32 codons to encode all 20 amino acids and encodes only 1 stop codon (amber codon). Applicant has used this approach to generate several cyclotide-based libraries using the loops 1, 2, 5 and/or 6 of cyclotide MCoTI-I as scaffold (**FIG. 4A**). These libraries showed a molecular complexity ranging from 10^6 (one loop) to 10^9 (2 loops). Expression of 60 of these clones revealed that around 70% of the sequences were able to express and adopt a native cyclotide fold when expressed in bacteria (**FIG. 4B**), as determined by biological activity and NMR [38, 41, 47]. This approach was also used for the generation of libraries using other loops and other cyclotide scaffolds such as kalata B1, which has been shown to have oral bioavailability [39]. Alternatively, the degenerate template can be also synthesized from mixtures of trinucleotide codons representing all 20 amino acids and no stop codons.

Cell-based screening FRET reporter

[0151] A FRET-based reporter can be used for the in-cell detection of cyclotides that inhibit a particular protein/protein interaction. As an example, the CyPet and YPet fluorescent proteins can be used as a FRET-couple to monitor protein interactions inside the cell [52-54]. The principle for this approach is depicted in **FIG. 5**. In order to facilitate the interaction between targeted domains and prevent any steric hindrance that will interfere with the molecular recognition process, addition of appropriate flexible polypeptide linkers (i.e. [GGG]_n) are added at the junctions between the interacting proteins or protein domains and the corresponding fluorescent proteins. Briefly, the N-terminus of soluble hBAFF (134-285, Δ 217-224) (this mutated version of soluble BAFF with eight residues at the flap region replaced by 2 Gly residues avoids oligomerization while still binding BAFF receptors [55]) and the extracellular Cys-rich domains (CRDs) of BAFF receptors hBR3 (1-62), hTACI (68-109) and hBCMA (1-54) are fused to either the C-terminus of CyPet and YPet, respectively. FRET reporters for murine BAFF and BAFF-receptors also can be generated as described above. BAFF and the BAFF receptor extracellular CRDs have shown to tolerate well the addition of protein domains (e.g., GST and/or thioredoxin) to their N or C-termini and can be expressed using bacterial expression systems without affecting their biological activity [55-57].

Cell-based screening/selection of cyclotide-based libraries for BAFF receptor antagonists

[0152] The cyclotide-based library is cloned into a pASK expression vector, while the FRET-based reporters are cloned into plasmids pRSF-duet and pBAD33, respectively. The library and reporter plasmids both contain different replication origins (ColE1, p15A or pRSF1030) to prevent replication dominance of one plasmid [58]. These plasmids also have different inducible bacterial promoters (arabinose, tetracycline or T7 promoters) in order to control the expression level of the library and screening reporter-system. The plasmids encoding the reporters are first transformed in Origami (DE3) competent cells to provide the corresponding FRET-based electrocompetent cell-line reporters. The library plasmid (with complexities $\approx 10^9$) are then transformed to into the corresponding cell-based reporters. Transformation conditions that ensure from 10- to 1000-fold coverage of the library are used. This usually requires performing between 20-50 transformations.

[0153] Screening and selection using the FRET-based reporter is performed in solution using fluorescence-activate cell sorting (FACS). Briefly, expression of the cyclotide-based peptide library is induced to “load” the cell with the potential antagonist. Expression of the fluorescent reporter protein-constructs is then induced. This order is important to allow the cyclization and folding of the cyclotide inside the bacterial cytoplasm before the screening can take place. As shown in **FIG. 6** (as an exemplar) for the RING-mediated Hdm2-HdmX interaction, FACS can easily separate the two FRET states. This selection process requires no more than 6 iterations to enrich the original library in cyclotide sequences that inhibit a particular target interaction. To minimize the selection of false positives in every round of selection, the selected pool of plasmids encoding cyclotides are re-transformed in fresh competent cells encoding the reporter. (See also **FIG. 6** wherein this approach to select novel cyclotides able to antagonize the RING-mediated interaction between Hdm2 and HdmX. The same protocol is used to enrich a population of *E. coli* cells from an initially predominant FRET-ON phenotype (complex BAFF-BAFF CRD receptor complex) into a FRET-OFF phenotype (complex BAFF-BAFF CRD receptor complex antagonized). The enrichment process is also monitored by PCR and DNA sequencing. This approach is used to select antagonists against individual BAFF receptor CDRs. To avoid cross reactivity with other individual receptors, an extra selection step is performed at the end of the evolution process

to avoid sequences able to antagonize to the other two BAFF CDR receptors. For example, selected sequences able to antagonize BAFF-BR3 (i.e. FRET-OFF phenotype) are used at the end of the evolution process screened against BAFF-TACI and BAFF-BCMA FRET-reporters to select cells expressing cyclotides with a FRET-ON phenotype, i.e. not able to cross-react with either TACI or BCMA.

[0154] It is important to note that the FRET-based reporter can be also used *in vitro* using the purified fluorescent constructs for the characterization of antagonists.

[0155] Based on typical hit rates for screens that target protein-protein interactions [59] and previous results using other protein targets, Applicant anticipates to have fewer than 200 different hits. Most of these sequences, however, belong to a small number of peptide families. DNA sequencing allows the extraction of sequence consensus. Representative members of these families are produced either by chemical or recombinant methods and characterized as described below.

Biophysical characterization of selected cyclotides

[0156] Selected cyclotides can be recombinantly produced using protein splicing units [41, 47] or chemically synthesized by solid-phase peptide synthesis (SPPS) using a GSH-induced “one-pot” cyclization/folding process as described in the literature [37, 41, 47]. Initial biophysical characterization of the antagonists includes confirming their ability to inhibit the protein complexes using a standard FRET-based assay (for IC₅₀ values) and/or fluorescence anisotropy (*K_d* values) as previously described [41]. It is important to highlight that the FRET-based reporter mentioned above can be also used *in vitro* using the purified fluorescent fusion constructs. Fluorescence anisotropy assays are performed using fluorescent-labeled cyclotides obtained by either chemical synthesis [37, 41] or recombinant expression [47] as previously described by Applicant. Characterization of the mode of inhibition can be performed by high-field nuclear magnetic resonance (NMR). NMR is a very powerful technique to map the interacting site of a peptide/protein with its partner. This structural information can also be used to identify key residues in the molecular interaction to help in the design of new biased libraries during the ligand maturation process of active cyclotides.

In vitro biological activity

[0157] The ability of selected cyclotides to antagonize an individual extracellular BAFF receptor CRD is tested *in vitro* using competitive BAFF-binding ELISA on BAFF receptor-expressing cells as previously described [60]. Briefly, BHK cells transfected with full length BR3, TACI or BCMA (2×10^5 /well) are seeded on Nunc round-bottom plates at 4° C and incubated with serial dilutions of active cyclotide and His-tagged BAFF (10 ng/mL) for 45 min in cell-binding buffer (PBS with 0.5% BSA and 1% FBS (PBF)). Cells are washed with PBF (3x) and the amount of cell-bound His-tagged BAFF quantified by ELISA. IC₅₀ values are calculated as the concentration of cyclotide that inhibits BAFF binding to 50% levels. Selected cyclotides are also tested in human or murine B cell proliferation assays as previously described in the literature [60]. Briefly, B cell proliferation assays are performed using murine or human B cells isolated from peripheral blood mononuclear cells by positive selection using CD19 MACS magnetic beads. To evaluate the antagonistic effects of selected cyclotides, B cells (1×10^5 /well) are incubated with soluble recombinant His-tagged BAFF (10 ng/mL) and in the presence and absence of various concentrations of active cyclotide. The same assay format, with the exception that BAFF is omitted, is used to assess potential agonistic effects. B-cell proliferation is analyzed at day 6 by adding Celltiter Glo (Promega). The plates are then read in a luminometer after a 10-min incubation at room temperature.

In vivo biological activity

[0158] The most active and stable cyclotide/s (and appropriate controls [38, 41]) are tested in SLE-prone NZM female mice. Pure folded cyclotide (≈ 200 -300 mg) is produced by solid-phase peptide synthesis using a one-pot cyclization folding reaction developed as described in the literature [37, 38, 41, 64]. This approach can be easily scaled up for the production of up to 400 mg of pure folded cyclotide [38, 41]. Briefly, mouse cohorts (n=10) are treated with vehicle (phosphate buffer saline, PBS) and the corresponding cyclotide at different doses once daily for up to 30 days by intravenous injection. Doses are determined based on the corresponding PK studies for the bioactive cyclotides. Without being bound by theory, doses are anticipated to range from 20 to 1 mg/kg. Health checks are performed daily to observe parameters such as body conditioning score, overall appearance and cleanliness, strength of grip, skin color and tone, mobility, gait, and activity level as indicators of potential drug

related toxicities. Individual weights are recorded thrice weekly, comparing the control and treatment groups as an additional indicator of tolerance of drug treatment as well as providing the average weight for calculation of drug dosing. At the end of the treatment and prior to euthanasia, the animals are evaluated for proteinuria, and sera will be collected for levels of total IgG and IgG autoantibodies. Kidneys are harvested from the euthanized mice and evaluated by histology and immunofluorescence as previously described [27].

Equivalents

[0159] It should be understood that although the present disclosure has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the disclosure embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this disclosure. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the disclosure.

[0160] The disclosure has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the disclosure with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0161] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[0162] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

Exemplary Sequences

1) Full sequence of soluble human BAFF

QVAALQGD LASLRAELQGHHAELKPAGAGAPKAGLEEAPAVTAGLKIFEPPAPGEGNSSQNSRNKRAVQGP
EETVTQDCLQLIADSETPTIQKGSYTFVPWLLSFKRGSAL EEEKENKILVKETGYFFIYGQVLYTDKTYAMGHLIQR
KKVHVFGDELSLVTLFRCIQNMPETLPNNSCYSAGIAKLEEGDELQLAIPRENAQISLDGDVTFFGALKLL
PETLPNNSCYSAGIAKLEEGDELQLAIPRENAQISLDGDVTFFGALKLL

2) Full sequence of soluble murine BAFF

QLAALQADLMSLRMELQSYRGSATPAAPGAPELTAGVKLLTPAAPQPHNSSRGHRNRRAFQGPETE QDVD
LSAPPAPCLPGCRHSQHDDNGMNLRNIIQDCLQLIADSDTPTIRKGT YTFVPWLLSFKRGNAL EEEKENKIVVR
QTGYFFIYSQVLYTDPIFAMGHVIQRKKVHVFGDELSLVTLFRCIQNMPKTL PNNSCYSAGIARLEEGDEIQLAI
PRENAQISRNGDDTFFGALKLL

3) Sequence of soluble monomeric murine BAFF

KLTEQDVDLSAPPAPCLPGCRHSQHDDNGMNLRNIIQDCLQLIADSDTPTIRKGT YTFVPWLLSFKRGNAL EEEKENKIVVR
QTGYFFIYSQVLYTDPIFAMGHVIQRKKGGSLVTLFRCIQNMPKTL PNNSCYSAGIARLEEGDEIQLAI
AIPRENAQISRNGDDTFFGALKLL

4) Murine BR3 extracellular

MGARRLRVRSQRSDSSVPTQC NQTECFDPLVRNCVSCELFHTPDTGHTSSLEPGTALQPQEGSALRPDVA

5) Human BR3 extracellular

MRRGPRSLRGRDAPAPTPCVPAECFDLLVRHCVACGLLRTPRPKPAGASSPAPRTALQPQESVGAGAGEAAL
PLPGLL

6) Murine BCMA extracellular

MAQQCFHSEYFDSLLHACKPCHLRCSNPPATCQPYCDPSVTSSVKGTYT

7) Human BCMA extracellular

MLQMAGQCSQNEYFDSLLHACIPCQLRCSSNTPPLTCQRYCNASVTNSVKGTNA

8) Murine TACI extracellular

MAMAFCPKDQYWDSSRKSCVSCALTCQRSQRTCTDFCKFINCRKEQGRYYDHLLGACVSCDSTCTQHPQQ
CAHFCEKRPRSQANLQPELGRPQAGEVEVRSDNSGRHQGSEHGPGRLSSDQLTLYC

9) Human TACI extracellular

MSGLGRSRRGGRSRVDQEERFPQGLWTGVAMRSCPEEQYWDPLLGT CSMCKTICNHQSQRTCAAFCRSL
CRKEQGKFYDHLLRDCISCASICGQHPKQCAYFCENKLRSPVNLPELRRQRSGEVENNSDNSGRYQGLEHRG
SEASPALPGLKLSADQVALVYS

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WHAT IS CLAIMED

1. A method for isolating a candidate BAFF receptor antagonist, comprising:
 - a) inserting into a cell one or more first expression vectors encoding a cyclotide comprising the candidate BAFF receptor antagonist, and wherein the first expression vectors further comprise a first origin of replication;
 - b) inserting into the cell a reporter vector encoding a BAFF ligand and a vector encoding one or more BAFF receptors, wherein each reporter vector is distinct from the first expression vector and comprises a distinct origin of replication from the first origin of replication;
 - c) inducing expression of the candidate antagonist;
 - d) inducing expression of the reporter receptor and the BAFF ligand; and
 - e) selecting cells wherein the candidate BAFF receptor antagonist binds to the BAFF receptor, thereby isolating the candidate BAFF receptor antagonist.
2. The method of claim 1, wherein the candidate BAFF receptor antagonist comprises a peptide sequence (ranging from about 4 to 30 residues) that specifically binds a BAFF receptor or a CDR of an antibody that specifically binds a BAFF receptor.
3. The method of claim 1 or 2, wherein step b) is performed before step a).
4. The method of claim 1, wherein the one or more BAFF receptors are selected from the group of: BR3, TAC1, or BCMA.
5. The method of claim 1, wherein the cell of step b) comprises a vector encoding a BR3 receptor and a plasmid encoding a TAC1 receptor.
6. The method of claim 1, wherein the cell of step b) comprises a vector encoding a BR3 receptor and a BCMA receptor.
7. The method of claim 1, wherein the cell of step b) comprises one or more vector encoding a BR3 receptor, a TAC1 receptor, or a BCMA receptor.
8. The method of claim 1, further comprising isolating BAFF receptor antagonist of step e) and further inserting the isolated candidate receptor antagonist into a second or

further system comprising a vector encoding a BAFF receptor that is different from the BAFF receptor of step b) and repeating steps c) through e).

9. The method of any preceding claim wherein the candidate receptor antagonist specifically binds the BAFF receptors BR3 and TACI, but not BCMA.
10. The method of claims 1-8, wherein the candidate receptor antagonist specifically binds the BAFF receptors BR3 and BCMA, but not TACI.
11. The method of any preceding claim, wherein the cells of steps a) and/or b) are prokaryotic or eukaryotic, optionally E. coli cells.
12. The method of any preceding claim, wherein the reporter of step b) comprises the components of a FRET-based system, optionally a CyPet and a YPet fluorescent protein pair.
13. The method of claim 12, wherein a linker is inserted at the junctions between the interacting proteins or protein domains and the corresponding fluorescent proteins, and optionally, wherein the linker comprises the amino acid sequence [GGS]_n, wherein n is an integer from 1 to 20.
14. The method of any preceding claim, wherein the expression vector of step a) is a pASK expression vector and/or the expression vector of the reporter of step b) comprises pRSF-det and pBAD33.
15. The candidate BAFF receptor antagonist isolated by the method of any one of claims 1 to 10.
16. The method of any one of claims 1-12, wherein the BAFF ligand comprises the soluble human BAFF (shBAFF) or a fragment thereof.
17. The method of claim 16, wherein the fragment comprises the N-terminus of soluble hBAFF, optionally 134-285, and further optionally delta 217-224.
18. The method of claim 17, wherein the shBAFF is a mutated shBAFF wherein eight residues at the flap region are replaced by 2 glycine amino acids.
19. The method of any preceding claim wherein at least one reporter plasmid encodes an extracellular cysteine-rich CDR BAFF receptor selected from the group of human or

murine BR3 (hBR3 or mBR3), human or murine BCMA (hBCMA or mBCMA), or human or murine TACI (hTACI or mTACI), and optionally wherein the receptor is fused to the C-terminus to one of the fluorescent pair, optionally CyPet or YPet.

20. The method of claim 19, wherein the hBR3 comprises amino acids 1-62.
21. The method of claim 19, wherein the hTACI comprises amino acids 68-109.
22. The method of claim 19, wherein the hBCMA comprises amino acids 1-54.
23. A candidate BAFF receptor antagonist isolated by any prior claim.
24. A method for inhibiting the interaction of BAFF with a BAFF receptor comprising contacting a cell or solution comprising the BAFF and the BAFF receptor with the candidate receptor antagonist of claim 23.
25. The method of claim 24, wherein the contacting is in vitro, ex vivo or in vivo.
26. A method for treating SLE comprising administering to a subject suffering from SLE an effective amount of the candidate BAFF receptor antagonist of claim 23.
27. The method of claim 26, wherein the subject is a mammal.
28. The method of claim 26, wherein the mammal is a human patient.

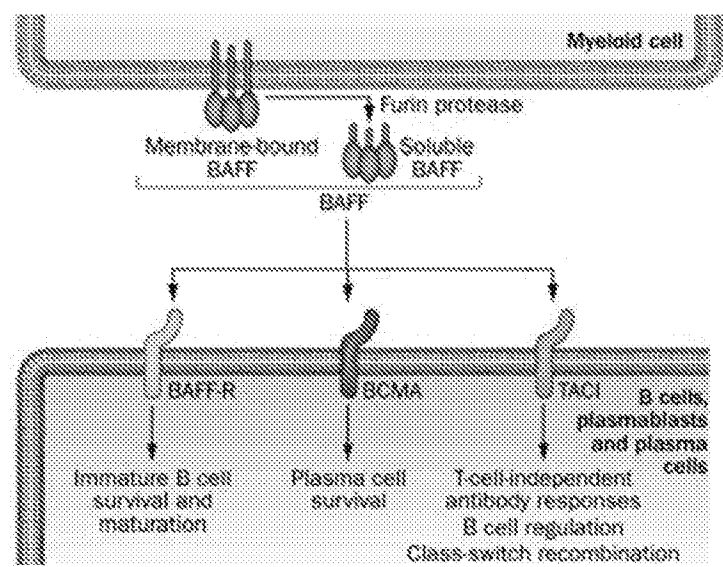


FIG. 1

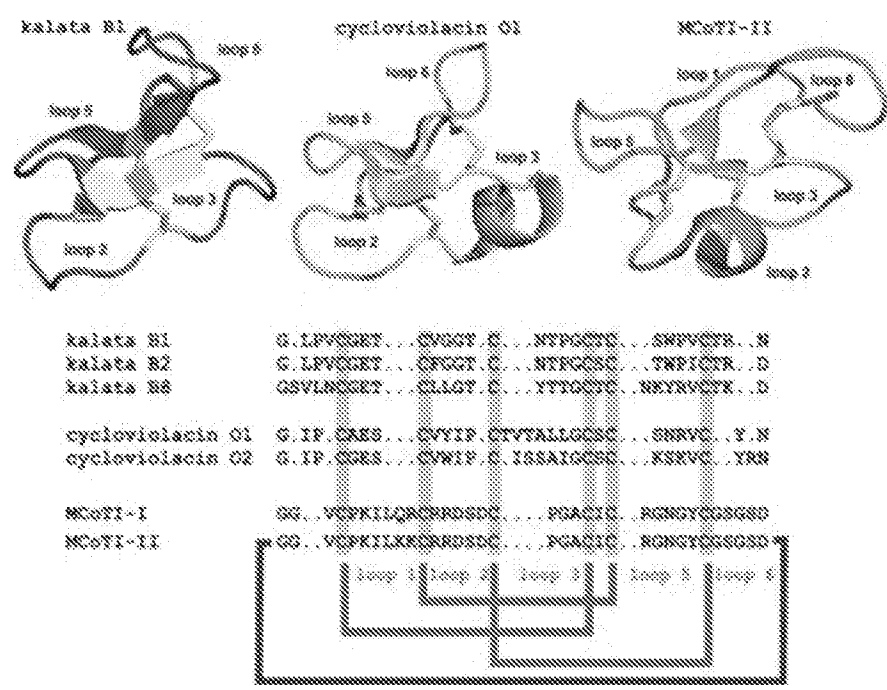
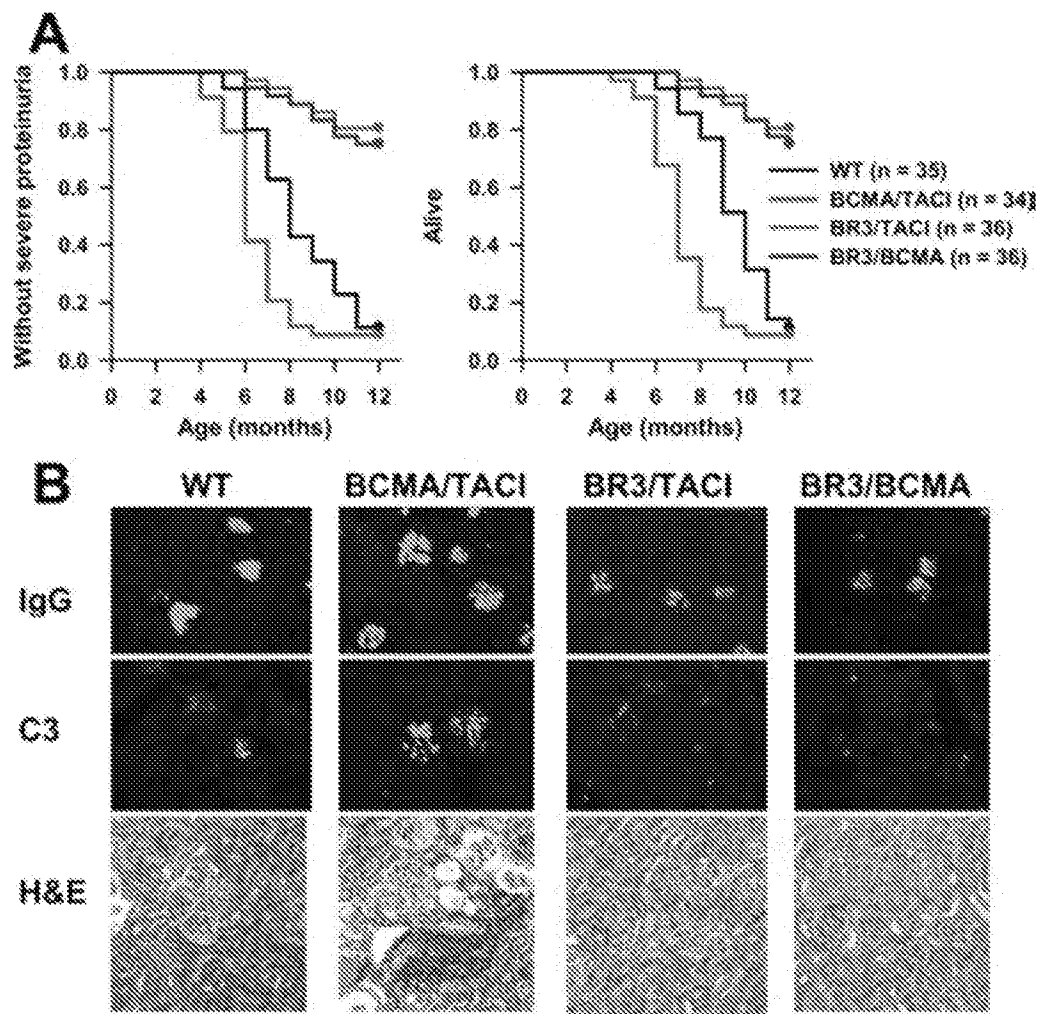
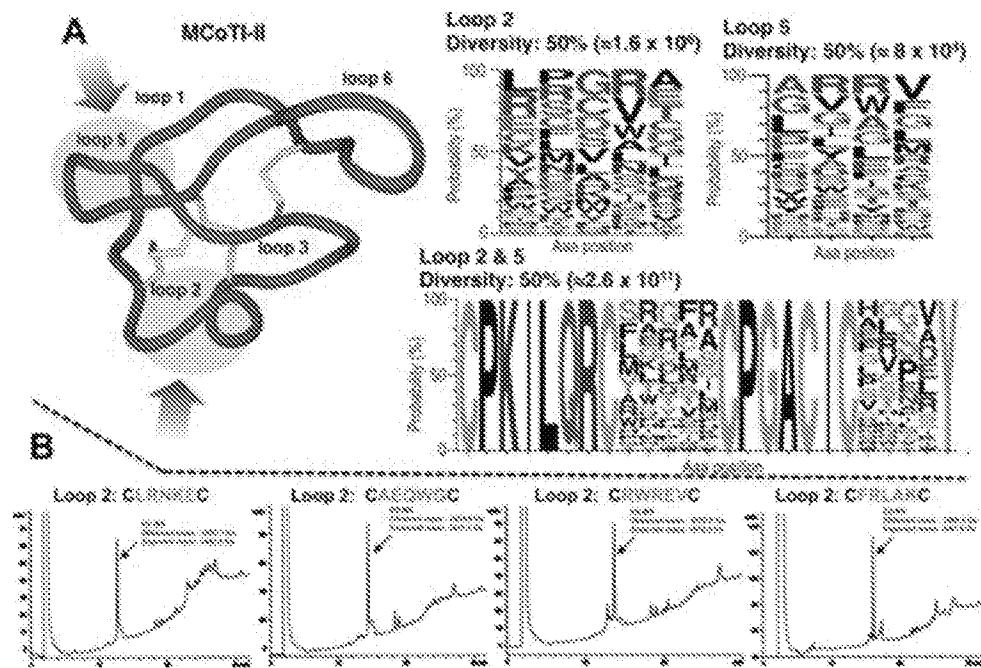


FIG. 2



FIGS. 3A – 3B



FIGS. 4A – 4B

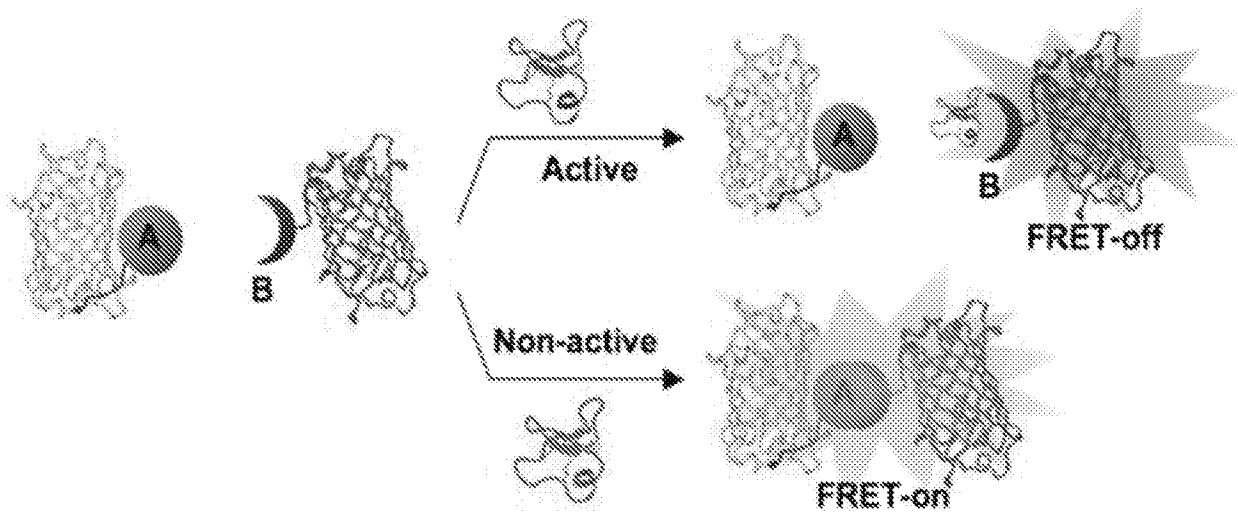
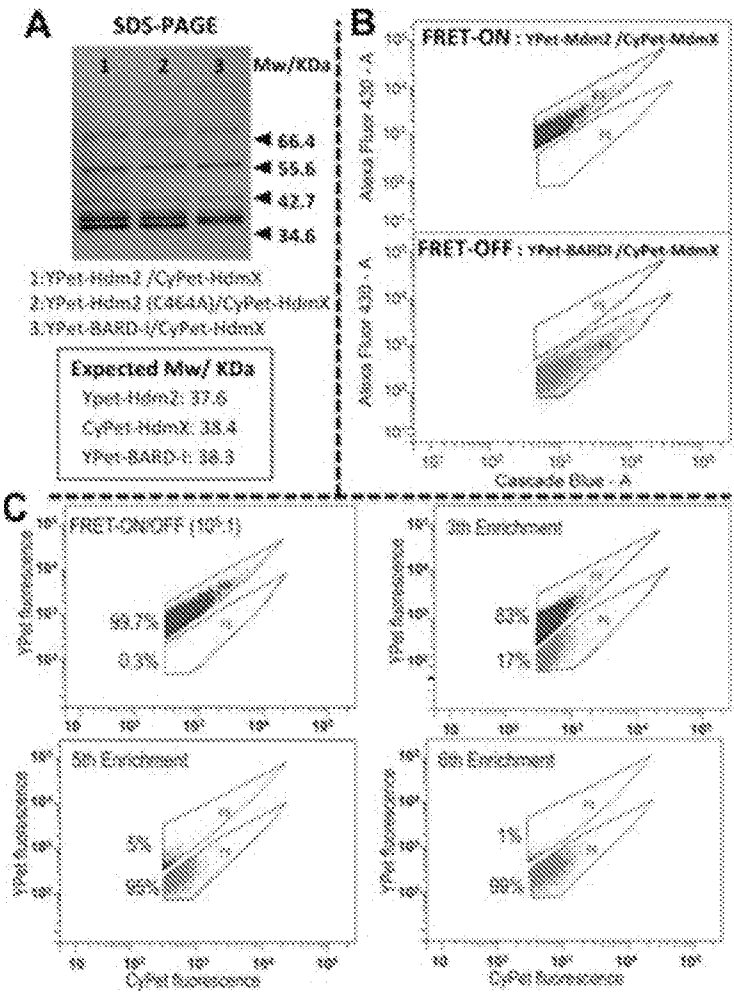


FIG. 5



FIGS. 6A – 6C