ANTIBODIES AND PROCESSES FOR PREPARING THE SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 61/054,047, filed May 16, 2008, which application is incorporated herein by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with the support of the United States government under Contract number R01 CA117802 by the National Cancer Institute (NCI) of the National Institute of Health (NIH).

BACKGROUND OF THE INVENTION

[0003] Traditional approaches to generate antigen specific monoclonal antibodies are limited. Typically only a small representation of the highly diverse antigen-specific B-cell population is obtained. In some cases antigen specific B-cells fail to proliferate due to self-tolerance. In other cases, antigen specific B-cells fail to proliferate after cell fusion with a myeloma fusion partner.

SUMMARY

[0004] Disclosed herein, in certain embodiments, are methods for generating, raising, obtaining and/or producing (for simplicity, the word "producing" as used herein is meant to equally refer to "generating," "raising," and "obtaining,"), a desired antibody are. In addition, systems, cell lines and organisms are described for use in producing a desired antibody. Further described are antibodies and compositions comprising such antibodies.

[0005] Provided herein are various methods for producing (a) antibody producing cells and (b) antibodies that overcome many of the problems associated with conventional antibody production.

[0005a] In one aspect, there is provided a method for producing a B-cell that produces an antibody that binds to a selected antigen comprising:

contacting a B-cell with

a selected antigen; and

a fusion peptide having a biological activity of MYC comprising a protein transduction domain, a MYC sequence, and at least two protein tags;

wherein the fusion peptide enters the nucleus of a cell and induces the B-cell to produce the antibody that specifically binds to the selected antigen, and

wherein the step of contacting the B-cell occurs *in vitro*.

[0005b] In one aspect, there is provided a method for producing a B-cell that produces an antibody that binds to a selected antigen comprising:

contacting a B-cell that comprises a selected antigen with a fusion peptide comprising

a protein transduction domain;

a MYC sequence; and

at least two protein tags;

wherein the fusion peptide has a biological activity of MYC, enters the nucleus of a cell, and induces the B-cell to produce the antibody that specifically binds to the selected antigen, and

wherein the step of contacting the B-cell occurs *in vitro*.

[0005c] In certain embodiments, provided herein are methods for producing (a) antibody producing cells and (b) antibodies derived from cells (e.g., mammalian cells) that are that over-express MYC. In some embodiments, over-expression of MYC is induced by contacting the cell with a small molecule, a biologic, a peptide, an antibody, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1. In some embodiments, the small molecule is an antagonist of Mxi-1. In some embodiments, the small molecule is an antagonist of MAD. In some embodiments, over-expression of MYC is induced by contacting the cell with a siRNA molecule for Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, provided herein is a method for (a) producing antibody producing cells and (b) antibodies derived from cells wherein MYC is over-expressed. In some embodiments, over-expression of MYC is induced by contacting the cell with a small molecule, a biologic, a peptide, an antibody, or a combination thereof. In some embodiments,

[0015] Provided herein is a method for producing a human or humanized antibody comprising: (a) providing an human cell; (b) isolating a human gene that encodes the antibody from the human cell; and (c) introducing (or otherwise providing to) the gene into an cell that comprises an transgenic MYC gene or a exogenous Myc peptide (e.g., a recombinant Myc peptide (e.g., a Myc fusion peptide as described herein)). In some embodiments, the human gene encodes human IgH (immunoglobulin heavy chain) and IgL (immunoglobulin light chain), wherein the IgH and IgL together form an antibody that specifically binds the selected antigen. In some embodiments, the cell (e.g., a mammalian cell) is a B-cell. In some embodiments, a method disclosed herein further comprises transplanting the cell (e.g., a mammalian cell) into an organism (e.g., a mouse). In some embodiments, the human gene isolated encodes a first antibody and a second antibody. In some embodiments, a method disclosed herein further comprises recovering the antibody from the cell (e.g., a mammalian cell). In some embodiments, the transgenic MYC DNA sequence comprises an inducible promoter or a B-cell-selective promoter. In some embodiments, the exogenous Myc peptide (e.g., a recombinant Myc peptide (e.g., a Myc fusion peptide as described herein)) is a Myc-ER fusion peptide. In some embodiments, the Myc peptide is a Myc-GR fusion peptide.

[0016] Provided herein is a method for producing a human or humanized antibody comprising: (a) introducing (or otherwise providing to) a gene encoding a human immunoglobulin into a cell that over-expresses Myc; and (b) isolating the encoded human immunoglobulin. In some embodiments, over-expression of MYC is induced by contacting the cell with a small molecule, a biologic, a peptide, an antibody, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1. In some embodiments, the small molecule is an antagonist of Mxi-1. In some embodiments, the small molecule is an antagonist of MAD. In some embodiments, over-expression of MYC is induced by contacting the cell with a siRNA molecule for Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, over-expression of MYC is induced by contacting the cell with a shRNA molecule for Max-1, Mxi-1, MAD, or a combination thereof.

[0017] Provided herein are isolated B-cells for producing a human or humanized antibody, wherein the B-cells over-expresses Myc. In some embodiments, over-expression of MYC is induced by contacting the cell with a small molecule, a biologic, a peptide, an antibody, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, the small molecule is an antagonist of Max-1. In some embodiments, the small molecule is an antagonist of Mxi-1. In some embodiments, the small molecule is an antagonist of MAD. In some embodiments, over-expression of MYC is induced by contacting the cell with a siRNA molecule for Max-1, Mxi-1, MAD, or a combination thereof. In some embodiments, over-expression of MYC is induced by contacting the cell with a shRNA molecule for Max-1, Mxi-1, MAD, or a combination thereof.

[0017a] In one aspect, there is provided a fusion peptide, comprising: (a) a protein transduction domain sequence; (b) a MYC sequence; (c) at least two protein tags; and (d) optionally, one or more molecules that link the protein transduction domain sequence and the MYC sequence;
wherein the fusion peptide is able to enter the nucleus of a cell and has the biological activity of MYC.

[0017b] In one aspect, there is provided a fusion peptide, wherein the peptide comprises SEQ ID NO:5.

[0017c] In one aspect, there is provided a peptide, wherein the peptide has the following sequence:
MRKKRRQRRRMDFFRVVENQQPPATMPLNVSFTNRNYDLDYDSVQPYFYCDEEENFYQQQQQ
SELQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPSYVAVTPFSLRGDNDGGGGSFSTADQLEMVT
ELLGGDMVNQSFICDPDDETFIKNIIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGH
SVCSTSSLYLQDLSAAASECIDPSVVFPYPLNDSSSPKSCASQDSSAFSPSSDSLLSSTESSPQGSPE
PLVLHEETPPTTSSDSEEEQEDEEEIDVVSVEKRQAPGKRSESGSPSAGGHSKPPHSPLVLKRCHV
STHQHNYAAPPSTRKDYPAAKRVKLDSVRVLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQR
RNELKRSFFALRDQIPELENNEKAPKVVILKKATAYILSVQAEEQKLISEEDLLRKRREQLKHKLE
QLRKGELNSKLEGKPIPNPLLGLDSTRTGHHHHHH.

[0017d] In one aspect, there is provided a composition comprising:
(a) any one of the fusion peptide disclosed herein; and
(b) one or more pharmaceutically acceptable excipients.

[0017e] Also provided herein are isolated

antibodies prepared according to the process of any of the methods disclosed herein. In one aspect, there is provided an antibody that binds to a selected antigen produced by any one of the methods disclosed herein. In another aspect, there is provided a pharmaceutical composition, comprising:

an antibody of the preceding aspect; and

one or more pharmaceutically acceptable carriers.

[0017f] And further provided herein are mammalian cells that have been engineered to produce a recombinant form of any antibody prepared according the process of the methods disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1. An illustrative example of surface phenotype of tumors and cell lines that arise in Eμ-*MYC*/BCR^{HEL}/sHEL transgenic mice. The black filled histograms correspond to the profiles of cells obtained from wild type mice, the grey trace corresponds to the BCR^{HEL} transgenic mice, the thin line or dotted trace correspond to BCR^{HEL}/sHEL mice, and the black trace correspond to the cells obtained from triply transgenic mice. The data represent the expression of the indicated markers on B220+ splenocytes.

[0019] Figure 2. An illustrative example of immunoglobulin production and HEL specific titers in tumors and cell lines that arise in Eμ-*MYC*/BCRHEL/sHEL mice. A defined number of cells (10^5) derived from each cell line (TBL-1, TBL-8, TBL-14 and TTLN9, all derived from the tumors that arose in Eμ-*MYC*/BCR^{HEL}/sHEL mice) were seeded in a 24 well plate, in 1ml of growth medium, without any added cytokines. Samples of the supernatant were collected 4 days later and assayed for the concentration of total IgM (panel A), as well as for the titer of HEL specific IgM (panel B). Sera from various mice were used to compare antibody production to the cell lines. These mice included wild type C57/BL6 mice (WT), BCR^{HEL} transgenic mice (BCR-tg), sHEL transgenic mice (Ag-tg), BCR^{HEL}/sHEL doubly transgenic mice (BCR/Ag-tg), Eμ-*MYC* mice and tumor-bearing Eμ-*MYC*/BCR^{HEL}/sHEL triply transgenic mice (BL). The results presented here are from one experiment, representative of three independent assays.

[0020] Figure 3. Appearance of activated B-cells following acute overexpression of MYC. Flow cytometric detection of activated B-cells. Analyses were performed on lymph node cells obtained from a wild type mouse (black filled histogram), a MMTV-tTA/TRE-MYC/BCRHEL/sHEL mouse that had been kept on doxycycline throughout (grey trace), and an MMTV-tTA/TRE-MYC/BCRHEL/sHEL mouse that had been taken off doxycycline a week prior to euthanasia (black trace). Cells were stained with antibodies to two molecules that are upregulated following the selected antigen-dependent activation of B-cells, CD69 (A), and B7-2 (CD86) (B). The traces represent the levels of CD69 and B7-2 present on the B220+ fraction of the cells, ascertained by gating on the Cychrome-C staining cells by flow cytometry.

[0021] Figure 4. Accumulation of activated B-cells requires the continuous overexpression of MYC. Activation of B-cells in response to MYC. The number of activated B-cells in lymph nodes was determined as described previously (34). Each data point in these graphs represents the number of activated B-cells detected in the lymph nodes of an individual mouse. Cohorts of four mice were used for each time point. This figure shows the requirement for MYC in the initiation and

Claims:

1. A fusion peptide, comprising:

(a) a protein transduction domain sequence;

(b) a MYC sequence;

(c) at least two protein tags; and

(d) optionally, one or more molecules that link the protein transduction domain sequence and the MYC sequence;

wherein the fusion peptide is able to enter the nucleus of a cell and has the biological activity of MYC.

2. The fusion peptide of claim 1, wherein the protein transduction domain sequence is a Tat protein transduction domain sequence.

3. The fusion peptide of claim 2, wherein the Tat protein transduction domain sequence is selected from the group consisting of $TAT_{[48-57]}$ and $TAT_{[57-48]}$.

4. The fusion peptide of any one of claims 1 to 3, wherein the two protein tags comprise one or more of a polyhistidine tag and an epitope tag.

5. The fusion peptide of claim 4, wherein the two protein tags are a 6-histidine tag and a V5 epitope tag.

6. The fusion peptide of any one of claims 1 to *5,* wherein the MYC sequence is 80 to 100% identical to the sequence of NCBI Accession Numbers NP002458.2.

7. A fusion peptide, wherein the peptide comprises SEQ ID NO:5.

8. A peptide, wherein the peptide has the following sequence:

MRKKRRQRRRMDFFRVVENQQPPATMPLNVSFTNRNYDLDYDSVQPYF
YCDEEENFYQQQQQSELQPPAPSEDIWKKFELLPTPPLSPSRRSGLCSPSYV
AVTPFSLRGDNDGGGGSFSTADQLEMVTELLGGDMVNQSFICDPDDETFI
KNIIIQDCMWSGFSAAAKLVSEKLASYQAARKDSGSPNPARGHSVCSTSSL
YLQDLSAAASECIDPSVVFPYPLNDSSSPKSCASQDSSAFSPSSDSLLSSTES
SPQGSPEPLVLHEETPPTTSSDSEEEQEDEEEIDVVSVEKRQAPGKRSESGS
PSAGGHSKPPHSPLVLKRCHVSTHQHNYAAPPSTRKDYPAAKRVKLDSVR
VLRQISNNRKCTSPRSSDTEENVKRRTHNVLERQRRNELKRSFFALRDQIP

ELENNEKAPKVVILKKATAYILSVQAEEQKLISEEDLLRKRREQLKHKLEQ LRKGELNSKLEGKPIPNPLLGLDSTRTGHHHHHH.

9. A composition comprising:

(a) the fusion peptide of any one of claims 1 to 8; and

(b) one or more pharmaceutically acceptable excipients.

10. A method for producing a B-cell that produces an antibody that binds to a selected antigen comprising:
contacting a B-cell with

a selected antigen; and

a fusion peptide having a biological activity of MYC comprising a protein transduction domain, a MYC sequence, and at least two protein tags;

wherein the fusion peptide enters the nucleus of a cell and induces the B-cell to produce the antibody that specifically binds to the selected antigen, and

wherein the step of contacting the B-cell occurs *in vitro*.

11. The method of claim 10, wherein the fusion peptide is a fusion peptide of any one of claims 2 to 8.

12. A method for producing a B-cell that produces an antibody that binds to a selected antigen comprising:

contacting a B-cell that comprises a selected antigen with a fusion peptide comprising

a protein transduction domain;

a MYC sequence; and

at least two protein tags;

wherein the fusion peptide has a biological activity of MYC, enters the nucleus of a cell, and induces the B-cell to produce the antibody that specifically binds to the selected antigen, and

wherein the step of contacting the B-cell occurs *in vitro*.

13. The method of claim 12, wherein the B-cell expresses the selected antigen.

14. The method of claim 12, wherein the fusion peptide is a fusion peptide of any one of claims 2 to 8.

15. The method of any one of claims 10 to 14, wherein the antibody is a human antibody.

16. The method of any one of claims 10 to 15, wherein the selected antigen is selected from the group consisting of: BAFFR, NGF, NGFR, IL-2, IL-10, IL-17, IL-24, RANTES, MIPla, MIP-lb, MCP-1, MIP-4, CXCR3, CXCR4, CCR3, CCR5, CCR9, HIV Env, and Influenza Hemagglutinin.

17. The method of any one of claims 10 to 15, wherein the selected antigen is a self antigen.

18. The method of any one of claims 10 to 17, wherein the selected antigen is a human antigen.

19. The method of any one of claims 10 to 18, wherein the B-cell is anergic.

20. The method of any one of claims 10 to 19, wherein the B-cell is a human B-cell.

21. The method of any one of claims 10 to 20, wherein the B-cell is a chimeric cell, with a human immunoglobulin locus.

22. The method of any one of claims 10 to 21, further comprising recovering the antibody that binds to the selected antigen.

23. An antibody that binds to a selected antigen produced by the method of claim 22.

24. A pharmaceutical composition, comprising:

an antibody of claim 23; and

one or more pharmaceutically acceptable carriers.