reported in US 2006/0051348. In WO 2008/144763 and WO 2008/045140 antibodies to IL-6 and uses thereof and a culture method for obtaining a clonal population of antigen-specific B-cells are reported, respectively. A culture method for obtaining a clonal population of antigen-specific B-cells is reported in US 2007/0269868. Masri, et al. (in Mol. Immunol. 44 (2007) 2101-2106) report the cloning and expression in E.coli of a functional Fab fragment obtained from single human lymphocyte against anthrax toxin. A method for preparing immunoglobulin libraries is reported in WO 2007/031550.

In WO 2009/10515 a method of making hybrid cells that express useful antibodies is reported. High affinity antibodies that neutralize Staphylococcus enterotoxin B are reported in WO 2008/085878. Amanna, I.J., and Slifka, M.K., report the quantitation of rare memory B cell populations by two independent and complementary approaches (J. Immunol. Meth. 317 (2006) 175-185). In WO 2011/052545 a method for producing antigen-specific B cell population is reported.

Summary ~~of the Invention~~

Herein is reported a new method for the rapid, efficient and reproducible generation of rabbit (or human) antibodies starting from rabbit (or human) B-cells and comprising at least one cultivation step.

Herein is further reported a feeder mix that can be used in combination with rabbit CD40L-expressing mammalian cells providing a highly efficient rabbit B-cell stimulation and cultivation system.

Herein is further reported a feeder mix that can be used in combination with human CD40L-expressing mammalian cells providing a highly efficient human B-cell stimulation and cultivation system.

~~One aspect a~~As reported herein is a method for co-cultivating rabbit B-cells and rabbit CD40L expressing mammalian cells.

~~One aspect a~~As reported herein is a method for co-cultivating rabbit B-cells and rabbit CD40L expressing mammalian cells in the presence of/together with IL-2 and IL-21. In one embodiment the rabbit CD40L expressing mammalian cell is a CHO or BHK cell.

~~One aspect a~~As reported herein is a method for co-cultivating human B-cells and human CD40L expressing mammalian cells.

~~One aspect a~~As reported herein is a method for co-cultivating human B-cells and human CD40L expressing mammalian cells in the presence of/together with IL-2 and/or IL-21 and/or IL-6.

In one embodiment the human CD40L expressing mammalian cell is a BHK or CHO cell.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-cultivating are all human interleukins.

~~One aspect a~~As reported herein is a method for producing a rabbit antibody comprising the step of cultivating an antibody secreting rabbit B-cell and rabbit CD40L expressing mammalian cells and recovering the antibody from the cultivation supernatant, thereby producing the rabbit antibody.

In one embodiment the rabbit CD40L expressing mammalian cell is a CHO or BHK cell.

One aspect as reported herein is a method for producing an antibody comprising the step of co-cultivating a rabbit B-cell with a rabbit CD40L expressing mammalian cell in the presence of IL-2 and IL-21.

~~One aspect a~~As reported herein is a method for producing a rabbit antibody comprising the step of cultivating one or more antibody secreting rabbit B-cells and rabbit CD40L expressing mammalian cells in the presence of/together with IL-2 and IL-21 and recovering the antibody from the cultivation supernatant, thereby producing the rabbit antibody.

In one embodiment the rabbit CD40L expressing mammalian cell is a CHO or BHK cell.

In one embodiment the IL-2 and IL-21 are added at the start of the cultivation.

In one embodiment the IL-2 and IL-21 are added solely at the start of the cultivation.

In one embodiment the rabbit B-cell is a naive or non-mature rabbit B-cell.

In one embodiment the B-cell is an IgG positive B-cell (IgG^+). IgG positive B-cells present the cell surface marker IgG which can be detected and labeled.

~~One aspect a~~As reported herein is a method for producing a human antibody comprising the step of cultivating an antibody secreting human B-cell and human CD40L expressing mammalian cells and recovering the antibody from the cultivation supernatant, thereby producing the human antibody.

In one embodiment the human CD40L expressing mammalian cell is a BHK or CHO cell.

~~One aspect a~~As reported herein is a method for producing a human antibody comprising the step of cultivating one or more antibody secreting human B-cells and human CD40L expressing mammalian cells in the presence of/together with IL-2 and/or IL-21 and/or IL-6 and recovering the antibody from the cultivation supernatant, thereby producing the human antibody.

In one embodiment the cultivating is in the presence of IL-2 and IL-21.

In one embodiment the cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the cultivating are all human interleukins.

In one embodiment the human CD40L expressing mammalian cell is a BHK or CHO cell.

In one embodiment the interleukins are added at the start of the cultivation.

In one embodiment the interleukins are added solely at the start of the cultivation.

In one embodiment the human B-cell is a mature human B-cell.

In one embodiment the B-cell is an IgG positive B-cell (IgG^+). IgG positive B-cells present the cell surface marker IgG which can be detected and labeled.

~~One aspect a~~As reported herein is a kit comprising rabbit CD40L expressing CHO cells and IL-2 and IL-21.

~~One aspect a~~As reported herein is a kit comprising human CD40L expressing BHK cells and IL-2 and/or IL-21 and/or IL-6.

In one embodiment the kit comprises IL-2 and IL-21.

In one embodiment the kit comprises IL-2 and IL-21 and IL-6.

In one embodiment the interleukins are all human interleukins.

One aspect as reported herein is a method for cultivating a rabbit B-cell secreting an antibody that specifically binds to a T-cell surface antigen and that mediates a negative stimulus to T-cells comprising the co-cultivation of the B-cell and a rabbit CD40L expressing mammalian cell in the presence of IL-2 and IL-21.

~~One aspect a~~As reported herein is a method for the co-cultivation of B-cells comprising the co-cultivation of B-cells and CD40L expressing mammalian cells in the presence of IL-2 or IL-21.

~~One aspect a~~As reported herein is a method for the co-cultivation of B-cells comprising the co-cultivation of B-cells and CD40L expressing mammalian cells in the presence of IL-2 and IL-21.

~~One aspect a~~As reported herein is a method for the co-cultivation of rabbit B-cells comprising the co-cultivation of rabbit B-cells and rabbit CD40L expressing mammalian cells in the presence of IL-2 and IL-21.

In one embodiment the mammalian cell is a CHO cell or a BHK cell.

~~One aspect a~~As reported herein is a method for the co-cultivation of B-cells comprising the co-cultivation of B-cells and CD40L expressing mammalian cells in the presence of IL-2 and/or IL-21 and/or IL-6.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21 and IL-6.

~~One aspect a~~As reported herein is a method for the co-cultivation of human B-cells comprising the co-cultivation of human B-cells and human CD40L expressing mammalian cells in the presence of IL-2 and/or IL-21 and/or IL-6.

~~In one embodiment the mammalian cell is a BHK cell or a CHO cell.~~

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-cultivating are all human interleukins.

~~One aspect a~~As reported herein is a method for cultivating B-cells secreting T-cell-inhibiting compounds comprising the co-cultivation of the B-cells and CD40L expressing mammalian cells.

~~One aspect a~~As reported herein is a method for the cultivation of antibody secreting B-cells comprising in the following order

i) a first co-cultivation of the B-cell and a CD40L expressing mammalian cell in the presence of a mitogenic stimulant, and
ii) a subsequent second co-cultivation of the B-cell and the CD40L expressing mammalian cell in the presence of an antibody production stimulant.

One aspect as reported herein is a method for the cultivation of an antibody secreting rabbit B-cell comprising in the following order

i) a first co-cultivation of the rabbit B-cell and a rabbit CD40L expressing mammalian cell in the presence of a mitogenic stimulant, and

ii) a subsequent second co-cultivation of the rabbit B-cell and the rabbit CD40L expressing mammalian cell in the presence of an antibody production stimulant.

In one embodiment the first and second co-cultivation are performed in the same cultivation vessel.

In one embodiment the method for the cultivation of antibody secreting B-cells comprises a co-cultivation of the B-cell and a CD40L expressing mammalian cell whereby at first a mitogenic stimulant is added to the cultivation and thereafter an antibody production stimulant is added to the cultivation.

In one embodiment the antibody production stimulant is added at least one hour after the mitogenic stimulant is added. In one embodiment the antibody production stimulant is added one to three days after the mitogenic stimulant is added to the cultivation.

In one embodiment the mitogenic stimulant is selected from the group comprising CD40- and CD40L-interacting compounds, ICOS- and ICOS-L-interacting compounds, APRIL, BAFF, CR2, CXCL9, CXCL12 (SDF-1), CXCL13, CXCL16, Flt-3L, Interleukin-1 (α/β), Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-7, Interleukin-10, Interleukin-14, Interleukin-21, SAP (signaling lymphocyte activation molecule [SLAM] associated protein), Staphylococcus A strain Cowan 1 particles (SAC; heat-killed, formalin-fixed), TLR Ligands such as LPS, different CpG ODNs or Resiquimod (R-848), TSLP, Tumor necrosis factor (TNF) alpha, type I Interferons (e.g. IFN α/β), and type II

interferon (e.g. IFNγ). B-cell activation might also be induced via anti-IgG, anti-CD20, and/or anti-CD27 antibodies.

In one embodiment the antibody production stimulant is selected from the group comprising CD40- and CD40L-interacting compounds, ICOS- and ICOS-L-interacting compounds, APRIL, BAFF, CR2, CXCL9, CXCL12 (SDF-1), CXCL13,

CXCL16, Flt-3L, Interleukin-1 (α/β), Interleukin-2, Interleukin-3, Interleukin-4, Interleukin-5, Interleukin-6, Interleukin-9, Interleukin-10, Interleukin-13, Interleukin-21, Interleukin-33, SAP (signaling lymphocyte activation molecule [SLAM] associated protein), Staphylococcus A strain Cowan 1 particles (SAC; heat-killed, formalin-fixed), TLR Ligands such as LPS, different CpG ODNs or Resiquimod (R-848), TSLP, Tumor necrosis factor (TNF) alpha, type I Interferons (e.g. IFNγ), and type II interferon (e.g. IFNγ). Further B-cell stimulation might also be induced via anti-IgG, anti-CD20, and/or anti-CD27antibodies.

In one embodiment the mitogenic stimulant is added at the beginning of the first co-cultivation.

In one embodiment the mitogenic stimulant is added 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after the beginning of the first co-cultivation.

In one embodiment the antibody production stimulant is added 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days after the addition of the mitogenic stimulant.

In one embodiment each of the co-cultivations is performed for 5 to 14 days.

In one embodiment the cultivating is for a total period of from 5 to 21 days. In one embodiment the cultivating is for a total period of from 6 to 14 days.

In one embodiment the mitogenic stimulant is removed before the beginning of the second co-cultivation. In one embodiment the removal is by exchange of the cultivation supernatant and or washing the cells with neutral media.

~~One aspect a~~As reported herein is a method for producing an antibody, which specifically binds to an antigen, comprising the following steps:

a) co-cultivating a pool of or a single deposited antibody secreting B-cell with CD40L expressing mammalian cells,
b) isolating a nucleic acid encoding the amino acid sequence of the variable light chain domain and isolating a nucleic acid encoding the amino acid sequence of the variable heavy chain domain of an antibody, which specifically binds to an antigen,
c) cultivating a cell comprising the nucleic acid isolated in b) or a variant thereof encoding a humanized version of the light and/or heavy chain variable domain within one or more expression cassettes,

d) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody, which specifically binds to an antigen.

One aspect as reported herein is a method for producing an antibody, which specifically binds to an antigen, comprising the following steps:

a) co-cultivating a pool of antibody secreting rabbit B-cells or a single antibody secreting rabbit B-cell with rabbit CD40L expressing mammalian cells in the presence of IL-2 and IL-21,
b) cultivating a cell comprising a nucleic acid encoding the variable regions or a humanized variant thereof of the antibody secreted by the rabbit B-cell co-cultivated in step a) within one or more expression cassettes,
c) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody, which specifically binds to an antigen.

In one embodiment the co-cultivating is in the presence of a mitogenic stimulant and/or an antibody production stimulant.

In one embodiment the method comprises the following steps:

a) providing a population of antibody secreting (mature) B-cells (obtained from the blood of an experimental animal or a human),
b) staining the cells of the population of B-cells with at least one fluorescence dye (in one embodiment with one to three, or two to three fluorescence dyes),
c) depositing single cells of the stained population of B-cells or a pool of cells from the stained population of B-cells in individual containers (in one embodiment is the container a well of a multi well plate),
d) cultivating the deposited individual B-cells in the presence of CD40L expressing mammalian cells and optionally IL-2 and/or IL-21 and/or IL-6,
e) determining the binding specificity of the antibodies secreted in the cultivation of the individual B-cells,
f) determining the amino acid sequence of the variable light and heavy chain domain of specifically binding antibodies by a reverse transcriptase PCR and nucleotide sequencing, and thereby obtaining a monoclonal antibody variable light and heavy chain domain encoding nucleic acid,
g) introducing the monoclonal antibody light and heavy chain variable domain encoding nucleic acid in an expression cassette for the expression of an antibody,
h) introducing the nucleic acid in a cell,
i) cultivating the cell and recovering the antibody from the cell or the cell culture supernatant and thereby producing an antibody, which specifically binds to an antigen.

In one embodiment the method comprises the following steps:

a) providing a population of antibody secreting (mature) rabbit B-cells,

b) staining the cells of the population of rabbit B-cells with at least one fluorescence dye (in one embodiment with one to three, or two to three fluorescence dyes),

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g) introducing the monoclonal antibody light and heavy chain variable domain encoding nucleic acid in an expression cassette for the expression of an antibody,
h) introducing the nucleic acid in a cell,
i) cultivating the cell and recovering the antibody from the cell or the cell culture supernatant and thereby producing an antibody, which specifically binds to an antigen.

In one embodiment the cultivating is in the presence of IL-2 and IL-21.

In one embodiment the cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-cultivating are all human interleukins.

In one embodiment the pool of cells comprises of from about 10 B-cells to about 1,000,000 B-cells. In one embodiment the pool comprises of from about 500 B-cells to about 100,000 B-cells. In one embodiment the pool comprises about 500 B-cells.

One aspect as reported herein is a method for producing an antibody, which specifically binds to an antigen, comprising the following steps:

a) co-cultivating a pool of antibody secreting human B-cells or a single antibody secreting human B-cell with human CD40L expressing mammalian cells in the presence of IL-2 and/or IL-21 and/or IL-6,
b) cultivating a cell comprising a nucleic acid encoding the variable regions or a variant thereof of the antibody secreted by the human B-cell co-cultivated in step a) within one or more expression cassettes,
c) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody, which specifically binds to an antigen.

~~One aspect a~~As reported herein is a method for producing an antibody, which specifically binds to an antigen, comprising the following steps:

a) co-cultivating a pool of antibody secreting rabbit B-cells or a single deposited antibody secreting rabbit B-cell with rabbit CD40L expressing mammalian cells and IL-2 and IL-21,
b) isolating a nucleic acid encoding the amino acid sequence of the variable light chain domain and isolating a nucleic acid encoding the amino acid sequence of the variable heavy chain domain of an antibody, which specifically binds to an antigen,
c) cultivating a cell comprising the nucleic acid isolated in b) or a variant thereof encoding a humanized version of the light and/or heavy chain variable domain within one or more expression cassettes,

d) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody, which specifically binds to an antigen.

In one embodiment the co-cultivating is in the presence of a mitogenic stimulant and/or an antibody production stimulant.

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f) determining the amino acid sequence of the variable light and heavy chain domain of specifically binding antibodies by a reverse transcriptase PCR and nucleotide sequencing, and thereby obtaining a monoclonal antibody variable light and heavy chain domain encoding nucleic acid,

g) introducing the monoclonal antibody light and heavy chain variable domain encoding nucleic acid in an expression cassette for the expression of an antibody,

h) introducing the nucleic acid in a cell,

i) cultivating the cell and recovering the antibody from the cell or the cell culture supernatant and thereby producing an antibody, which specifically binds to an antigen.

In one embodiment the pool of cells comprises of from about 10 B-cells to about 1,000,000 B-cells. In one embodiment the pool comprises of from about 500 B-cells to about 100,000 B-cells. In one embodiment the pool comprises about 500 B-cells.

~~One aspect a~~As reported herein is the use of a rabbit CD40L expressing mammalian cell in the co-cultivation of antibody secreting rabbit B-cells.

One aspect as reported herein is the use of a rabbit CD40L expressing mammalian cell, IL-2 and IL-21 in the co-cultivation of antibody secreting rabbit B-cells.

In one embodiment the mammalian cell is a CHO cell.

In one embodiment the rabbit CD40L has the amino acid sequence of SEQ ID NO: 01.

~~One aspect a~~As reported herein is the use of a human CD40L expressing mammalian cell in the co-cultivation of antibody secreting human B-cells.

In one embodiment the mammalian cell is a BHK cell.

In one embodiment the human CD40L has the amino acid sequence of SEQ ID NO: 02.

~~One aspect a~~As reported herein is the use of SAC in the co-cultivation of antibody secreting B-cells and CD40L expressing mammalian cells.

One aspect as reported herein is the use of IL-2 and/or IL-21 for the co-cultivation of rabbit B-cells with feeder cells.

One aspect as reported herein is the use of SAC in the co-cultivation of antibody secreting B-cells optionally with CD40L expressing mammalian cells and/or IL-2 and/or IL-21 and/or IL-6.

~~One aspect a~~As reported herein is the use of a rabbit CD40L expressing mammalian cell and IL-2 and IL-21 in a co-culture of a rabbit B-cell.

~~One aspect a~~As reported herein is the use of a human CD40L expressing mammalian cell, and IL-2 and/or IL-21 and/or IL-6 in a co-culture of a human B-cell.

In one embodiment the co-culture is in the presence of IL-2 and IL-21.

In one embodiment the co-culture is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-culture are all human interleukins.

One aspect as reported herein is the use of IL-2 and/or IL-21 and/or IL-6 for the co-cultivation of human B-cells with feeder cells.

One aspect as reported herein is the use of a human CD40L expressing mammalian cell, IL-2 and/or IL-21 and/or IL-6 in the co-cultivation of antibody secreting human B-cells.

~~One aspect a~~As reported herein is a method for co-cultivating rabbit B-cells comprising the step of cultivating a single deposited rabbit B-cell or a pool of rabbit B-cells with rabbit CD40L expressing mammalian cells in the presence of IL-2 and IL-21.

In one embodiment of all aspects as reported herein the IL-2 is either human IL-2 or mouse IL-2 and the IL-21 is either human IL-21 or mouse IL-21.

One aspect as reported herein is a method for producing an antibody comprising the step of co-cultivating a human B-cell with a human CD40L expressing mammalian cell in the presence of IL-2 or IL-21 or IL-6 or a combination thereof.

One aspect as reported herein is a method for cultivating a human B-cell secreting an antibody that specifically binds to a T-cell surface antigen and that mediates a negative stimulus to T-cells comprising the co-cultivation of the human B-cell and a human CD40L expressing mammalian cell in the presence of IL-2 and/or IL-21 and/or IL-6.

~~One aspect a~~As reported herein is a method for co-cultivating human B-cells comprising the step of cultivating a single deposited human B-cell or a pool of human B-cells with human CD40L expressing mammalian cells in the presence of IL-2 and/or IL-21 and/or IL-6.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21.

In one embodiment the co-cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-cultivating are all human interleukins.

~~One aspect a~~As reported herein is the use of a rabbit CD40L expressing mammalian cell, IL-2 and IL-21 in a co-culture of a rabbit B-cell for improving cell growth.

~~One aspect a~~As reported herein is the use of a human CD40L expressing mammalian cell, IL-2 and/or IL-21 and/or IL-6 in a co-culture of a human B-cell for improving cell growth.

In one embodiment the co-culture is in the presence of IL-2 and IL-21.

In one embodiment the co-culture is in the presence of IL-2 and IL-21 and IL-6.

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In one embodiment the interleukins used in the co-cultivating are all human interleukins.

~~One aspect a~~As reported herein is a method for improving cell growth of rabbit B-cells comprising the step of cultivating a single deposited rabbit B-cell or a pool of rabbit B-cells with rabbit CD40L expressing mammalian cells in the presence of IL-2 and IL-21.

In one embodiment of all aspects as reported herein the IL-2 is either human IL-2 or mouse IL-2 and the IL-21 is either human IL-21 or mouse IL-21.

~~One aspect a~~As reported herein is a method for improving cell growth of human B-cells comprising the step of cultivating a single deposited human B-cell or a pool of human B-cells with human CD40L expressing mammalian cells in the presence of IL-2 and/or IL-21 and/or IL-6.

In one embodiment the cultivating is in the presence of IL-2 and IL-21.

In one embodiment the cultivating is in the presence of IL-2 and IL-21 and IL-6.

In one embodiment the interleukins used in the co-cultivating are all human interleukins.

Detailed Description of the Invention

The subject matter reported herein provides a generally applicable method for the rapid, efficient and reproducible generation of rabbit (or human) antibodies starting from rabbit (or human) B-cells and comprising at least one cultivation step.

It has been found that the addition of a rabbit CD40L expressing mammalian cell and IL-2 and IL-21 can improve the growth characteristics of rabbit B-cells, i.e. the rabbit B-cells, either a single deposited cell or as pool of cells, can be grown more rapidly to high cell densities than in the absence of the rabbit CD40L expressing mammalian cell and IL-2 and IL-21. Thus, it is possible to obtain high rabbit B-cell densities and correspondingly high IgG concentrations in the cultivation supernatant in short time.

Herein is reported as one aspect a CHO cell expressing rabbit CD40L.

One aspect as reported herein is the use of a rabbit CD40L expressing CHO cell together with IL-2 and IL-21 in the co-cultivation of rabbit B-cells.