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

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.

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~~ BHK or CHO cell in the presence of IL-2 and IL-21.

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 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.~~

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.

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.

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.

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.

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.

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.

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

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.

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.~~

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,

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.

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.

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.

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.~~

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.~~

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.~~

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.

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.

Patent Claims

1. A method for producing an antibody comprising the step of co-cultivating a rabbit B-cell with a rabbit CD40L expressing ~~mammalian~~ BHK or CHO cell in the presence of IL-2 and IL-21.

2. The method according to claim 1, characterized in that the B-cell is a non-mature B-cell.

3. The method according to any one of the preceding claims, characterized in that the IL-2 is human IL-2 and the IL-21 is murine IL-21.

4. The method according to any one of the preceding claims, characterized in that the B-cell is a single deposited B-cell.

5. The method according to any one of the preceding claims, characterized in that the B-cells are IgG positive B-cells (IgG^+ B-cells).

~~6. Use of IL-2 and/or IL-21 for the co-cultivation of rabbit B-cells with feeder cells.~~

~~7. The use according to claim 6, characterized in that the feeder cell is a rabbit CD40L expressing mammalian cell.~~

8. ~~A~~ The method ~~for cultivating a rabbit B-cell secreting an~~ according to any one of the preceding claims, wherein the 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~~.

9. ~~A~~ The method ~~for the cultivation of an antibody secreting rabbit B-cell~~ according to any one of claims 1 to 6 comprising in the following order

   i) a first co-cultivation of the rabbit B-cell and a rabbit CD40L expressing ~~mammalian~~ BHK or CHO 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.

~~108~~. The method according to claim ~~97~~, characterized in that 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).

~~119~~. The method according to any one of claims ~~9-7~~ or ~~108~~, characterized in that 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. IFN α/β), and type II interferon (e.g. IFNγ).

~~1210~~. ~~A~~ The method ~~for producing an antibody, which specifically binds to an antigen,~~according to any one of claims 1 to 6 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~~ BHK or CHO 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.

~~13~~11. The method according to claim ~~12~~10, characterized in that the method comprises the following steps:

a) providing a population of antibody secreting (mature) rabbit B-cells (obtained from the blood of an experimental animal or a human),
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),
c) depositing single cells of 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 rabbit B-cells in the presence of rabbit CD40L expressing ~~mammalian~~ BHK or CHO cells and IL-2 and IL21,
e) determining the binding specificity of the antibodies secreted in the cultivation of the individual rabbit 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 or a variant thereof encoding a humanized version of the light and/or heavy chain variable domain 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.

~~14. Use of a rabbit CD40L expressing mammalian cell, IL-2 and IL-21 in the co-cultivation of antibody secreting rabbit B-cells.~~

~~15~~12. ~~Use of SAC in the co-cultivation of~~The method according to any one of the preceding claims, wherein antibody secreting rabbit B-cells ~~optionally~~ are co-cultivated with CD40L expressing ~~mammalian~~ BHK or CHO cells and~~/or~~ IL-2 and~~/or~~ IL-21 and~~/or~~ IL-6 and SAC.

16. 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.

17. The method according to claim 16, characterized in that the B-cell is a non-mature B-cell.

18. The method according to any one of claims 16 to 17, characterized in that the co-cultivation is in the presence of IL-2, or IL-2 and IL-21, or IL-2 and IL-21 and IL-6.

19. The method according to any one of claims 16 to 18, characterized in that the B-cell is a single deposited B-cell.

20. The method according to any one claims 16 to 19, characterized in that the B-cells are IgG positive B-cells (IgG^+ B-cells).

21. Use of IL-2 and/or IL-21 and/or IL-6 for the co-cultivation of human B-cells with feeder cells.

22. The use according to claim 21, characterized in that the feeder cell is a human CD40L expressing mammalian cell.

23. The use according to any one of claims 21 to 22, characterized in that IL-2, or IL-2 and IL-21, or IL-2 and IL-21 and IL-6 are used.

24. 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.

25. 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.~~

~~26. The method according to claim 25, characterized in that the method comprises the following steps:~~

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

~~b) staining the cells of the population of human 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 in individual containers (in one embodiment is the container a well of a multi well plate),~~

~~d) cultivating the deposited individual human B-cells in the presence of human CD40L expressing mammalian cells and IL-2 and/or IL21 and/or IL-6,~~

~~e) determining the binding specificity of the antibodies secreted in the cultivation of the individual human 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.~~

~~27. The method according to any one of claims 25 to 26, characterized in that the co-cultivation is in the presence of IL-2, or IL-2 and IL-21, or IL-2 and IL-21 and IL-6.~~

~~28. 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.~~

~~29. The use according to claim 28, characterized in that IL-2, or IL-2 and IL-21, or IL-2 and IL-21 and IL-6 are used.~~