With this cytokine composition the inventors were able to determine a novel method for the preparation of population of antigen edited lymphocytes. Therefore, in another aspect, the present invention refers to a method of preparing a population of clinically relevant lymphocytes, comprising the steps of providing an isolated body sample of a mammal in particular a tissue sample or body liquid sample, comprising at least one lymphocyte and optionally separating the cells in the body sample, culturing the body sample *in-vitro* to expand and/or stimulate lymphocytes in the sample wherein the culturing comprises using IL-2, IL-15 and IL-21 wherein the concentration of IL-2 in the culture is in the range of 500 to 2000 U/ml; the concentration of IL-15 is in the range of 0.1 to 100 ng/ml and the concentration of IL-21 is in the range of 0.1 to 100 ng/ml, and optionally determining the presence of clinically relevant lymphocyte in the cultured sample ; wherein the *in-vitro* culturing comprises a first expansion step comprising an incubation in culture medium comprising IL-2, IL-15 and IL-21 until lymphocytes become detectable. In one example, there is disclosed a method of preparing a population of clinically relevant lymphocytes, comprising the steps of: obtaining a body sample from a mammal, in particular a tissue sample or body liquid sample, comprising at least one lymphocyte and optionally separating the cells in the body sample, culturing the body sample *in-vitro* to expand and/or stimulate lymphocytes in the sample, wherein the culturing comprises using IL-2, IL-15 and/or IL-21, and optionally determining the presence of clinically relevant lymphocyte in the cultured sample.

The method as disclosed herein leads to the formation of population of lymphocytes, which includes a population of clinically relevant lymphocytes. In one example, there is disclosed a clinically relevant lymphocyte obtained by the method disclosed herein wherein the clinically relevant lymphocyte is selected from a B-cell, an NK cell and T-cell. In a further example, there is disclosed a population of lymphocytes obtained the method as disclosed herein a population of clinically relevant lymphocytes.

The population of clinically relevant lymphocytes obtained with the method as disclosed herein is in particular advantageous for cellular immunotherapy. Thus, in another aspect, the present invention refers to a composition as described herein for use in medical treatment, in particular for treating or preventing an infectious disease, an autoimmune disease or a tumor disease, wherein the use comprises the generation of a population of clinically relevant lymphocytes with the method as disclosed herein. In yet another aspect, the present invention refers to Population of lymphocytes obtained by a method according to any of claims 4 to 15 comprising a population of clinically relevant lymphocytes, characterized by the following features: the percentage of T_{reg} based on the total number of T cells is below

C l a i m s

1. Composition for expanding lymphocytes comprising interleukin 2 (IL-2), interleukin 15 (IL-15) and interleukin 21 (IL-21), wherein the composition is in liquid form, the concentration of IL-2 in the liquid composition is in the range from 500 to 2000 U/ml, the concentration of IL-15 is in the range from 0.1 to 100 ng/ml and wherein the concentration of IL-21 is in the range from 0.1 to 100 ng/ml.

2. Composition according to claim 1, wherein the concentration of IL-2 in the liquid composition is in the range from 800 to 1200 U/ml.

3. Composition according to claim 2, wherein the concentration of IL-15 is in the range from 2 to 50 ng/ml, more preferably in the range from 5 to 20 ng/ml and wherein the concentration of IL-21 is in the range from 2 to 50 ng/ml, more preferably in the range from 5 to 20 ng/ml.

4. Method of preparing a population of clinically relevant lymphocytes, comprising the steps of:
   - providing an isolated body sample of a mammal, in particular a tissue sample or body liquid sample, comprising at least one lymphocyte and optionally separating the cells in the body sample;
   - culturing the body sample *in-vitro* to expand and/or stimulate lymphocytes in the sample wherein the culturing comprises using IL-2, IL-15 and IL-21 wherein the concentration of IL-2 in the culture is in the range of 500 to 2000 U/ml; the concentration of IL-15 is in the range of 0.1 to 100 ng/ml and the concentration of IL-21 is in the range of 0.1 to 100 ng/ml;
   - and optionally determining the presence of clinically relevant lymphocyte in the cultured sample;

   wherein the *in-vitro* culturing comprises a first expansion step comprising an incubation in culture medium comprising IL-2, IL-15 and IL-21 until lymphocytes become detectable.

5. Method according to claim 4, wherein the clinically relevant lymphocytes are selected from tumor-reactive lymphocytes, pathogen reactive lymphocytes and autoimmune reactive lymphocytes, preferably tumor-reactive lymphocytes.

6. Method according to claim 4 or 5, wherein the body sample is selected from the group consisting of peripheral blood, cord blood, bone marrow, lymph nodes liver, pleural effusion, thorax, abdominal cavity, synvial fluid, peritoneum, retroperitoneal space, thymus, and tumor, preferably peripheral blood or tumor.

7. Method according to any of claims 4 to 6, the time of incubation of the first expansion step is in the range from 6 hours to 180 days preferably in the range from 4 to 10 days, more preferably in the range from 6 to 8 days, most preferably about 7 days.

8. Method according to claim 7, wherein the *in-vitro* culturing comprises a second expansion step comprising an incubation in culture medium comprising feeder cells and/or an antibody against CD3 in addition to IL-2, IL-15 and IL-21.

9. Method according to claim 8, wherein the ratio of feeder cells to lymphocytes is in the range from 1:1 to 1:100, preferably in the range from 1:2 to 1 :50, more preferably in the range from 1:5 to 1:20, most preferably about 1:10.

10. Method according to any of claims 7 to 9, wherein the first expansion step comprises adding at least one of IL-2, IL-15 and IL-21 separately at a different time point to the cell culture, preferably, first IL-21 is added and in particular IL-15 is added second before adding IL-2.

11. Method according to any of claims 7 to 10, wherein the culture medium of the first and/or second expansion step comprises least one expansion antigen.

12. Method according to claim 11, wherein expansion antigen is a fragment of TAA, in particular a peptide comprising at least eight continuous amino acids of an amino acid sequence that is at least 80 % identical to the amino acid

sequences SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

13. Method according to any of claims 7 to 11, wherein the in-vitro culturing further comprises a refocusing step, comprising an incubation in culture medium comprising refocusing cells wherein preferably the time of the refocusing step is in the range from 1 to 6 days, preferably 1 to 3 days.

14. Method according to any of claims 4 to 13, wherein the testing for the presence of clinically relevant lymphocytes comprises using evaluation antigens and the evaluation antigens are presented to the cultured sample in a form selected from cells, in particular tumor cells, derived from the same mammal as the cultured sample (autologous cells), at least partially genetically matched allogeneic cells, in particular tumor cells, or cells expressing the clinically relevant antigens as a transgene.

15. Method according to claim 14, wherein the testing for the presence of clinically reactive lymphocytes comprises contacting the lymphocytes with at least one clinically relevant antigen and determining a change in either one of cytokine production, in particular INFγ production, cell proliferation, cytotoxicity, signaling and/or intracellular phosphorylation.

16. Composition according to any of claims 1 to 3, for use in medical treatment, in particular for treating or preventing an infectious disease, an autoimmune disease or a tumor disease, wherein the use comprises the generation of a population of clinically relevant lymphocytes with the method according to any of claims 4 to 15.

17. Population of lymphocytes obtained by a method according to any of claims 4 to 15 comprising a population of clinically relevant lymphocytes, characterized by the following features:

- the percentage of T_{reg} based on the total number of T cells is below 5 %, preferably below 3 %;
- the percentage of T_{H1}-cells based on the total number of T_H-cells is at least 50 %, preferably at least 70 %, more preferably at least 80 %,

- the percentage of CXCR3+ T-cells based on the total number of $CD8^+$ T-cells is at least 50 %, preferably at least 70 %, more preferably at least 80 %,
- the percentage of 4-1BB+ T-cells based on the total number of T-cells is at least 1 %, preferably at least 2 %, more preferably at least 2.5 %,
- the percentage of CD117+ T-cells based on the total number of T-cells is at least 1 %, preferably at least 2 %, more preferably at least 2.5 %,
- the percentage of CD3+CD4-CD8-cells based on the total number of T-cells is at least 1 %, preferably at least 3 %, more preferably at least 5 %; and
- the percentage of γδT-cells based on the total number of T-cells is at least 1 %, preferably at least 3 %, more preferably at least 5 %.