

A method for large-scale expansion of natural killer (NK) cells in a cell culture medium

Filed of the Invention

The invention relates to a method for large scale expansion of natural killer (NK) cells in a cell culture medium

Background of the Invention

Natural Killer (NK) cells are innate immune system lymphocytes which effectively control viral infection and many tumors in human body. The benefits of NK cells have been explored through various studies. They also function as regulatory cells involving immune responses. NK cells thus offer unique opportunities for new and improved therapeutic manipulation, either in vivo or in vitro, in a variety of human diseases. To date, NK cells manipulation has been used in hematopoietic and solid organ transplantation, promote antitumor immunotherapy and control inflammatory and autoimmune disorders.

However, the low numbers of these cells, for example in peripheral blood mononuclear cells, (PBMC) have resulted in various approaches to preferentially expand primary NK cells. Different methods have been disclosed in the prior arts including the disclosure by Wagner, et. al, 2017 regarding the addition of interleukin 2 (IL-2) for co-stimulating the expansion of purified NK cells from allogeneic donors. The prior arts including the Expansion of cytotoxic natural killer cells using irradiated autologous peripheral blood mononuclear cells and anti-CD16 antibody by Hong-Rae Lee, et. al., 2017 also disclosed the use of an anti-CD16 monoclonal antibody (mAb) and irradiated autologous peripheral blood mononuclear cells (PBMCs) (IrAPs) to provide a suitable environment (activating receptor-ligand interactions) for the NK cell expansion.

The prior arts still have limitations in terms of scope of the expansion and the further studies and improvement on a possible method is needed.

Limitation of the NK cells expansion technics that have been reported in prior arts include the presence of feeder cells in cell culture system and the moderate to low final NK cell purity. Although the purity of final cell product can be improved by using various cell sorting approaches, those approaches, however, require the necessarily use of an expansive cell sorting materials.

Summary of the Invention

In the present disclosure, the objective of the invention is to improve the existing methods for large scale expansion of natural killer (NK) cells. A method for large scale expansion of natural killer (NK) cells in a cell culture medium according to this invention comprises the steps of adding said cells to a closed system comprising a growth medium supplemented with serum is selected from the group consisting of human serum and autologous plasma; adding an effective concentration of CRX 527; expanding said cells within said system with heating wherein the said effective concentration of CRX 527 in the said growth medium is in the range of 0.01 µg/ml–10 µg/ml, preferably 100 ng/ml and the ratio between the cell density and the total volume of growth medium is in the range of 1.5 to 2.

In accordance with one aspect of the present invention, the cells are incubated for at least about 7 days. The culture system could be presented in the form of a pre-sterile bag or a bioreactor.

A suspension of natural killer (NK) cells obtained from any of the above claims wherein at least 70 %, preferably more than 80 % of the expanded cell population comprises activated NK cells.

Brief Descriptions of the Drawings

Figure 1 shows the proportions of NK cells (CD3-CD56+ cells) produced in indicated concentrations of CRX527 treated groups.

Figure 2 shows final cell number and the proportion of NK and T cells after expand CBNK cells in the said condition.

Detailed Descriptions of the Invention

The descriptions in more detail of this invention will be provided by way of example in order to make the descriptions clearer. The said descriptions in more detail, however, are provided without any limitation to the scope of the invention.

In this regard, this invention can be implemented or applied in various manners. This disclosure will present the different examples as references for implementations or applications having the same characteristics. Nevertheless, as mentioned above, these are provided without any limitation to the scope of the invention.

A method for large scale expansion of natural killer (NK) cells in a cell culture medium according to this invention refers to the method for large scale expansion of natural killer (NK) cells, particularly cord blood-derived NK Cells or CBNK cells which comprises the steps of adding said cells to a closed system comprising a growth medium supplemented with serum is selected from the group consisting of human serum and autologous plasma; adding an effective concentration of CRX 527; expanding said cells within said system with heating wherein the said

effective concentration of CRX 527 in the said growth medium is in the range of 0.01 µg/ml –10 µg/ml, preferably 100 ng/ml and the ratio between the cell density and the total volume of growth medium is in the range of 1.5 to 2.

In accordance with one aspect of the present invention, the cells are incubated for at least about 7 days. The culture system could be presented, but not limited to, in the form of a pre-sterile bag or a bioreactor.

A suspension of natural killer (NK) cells obtained from any of the above claims wherein at least 70 %, preferably more than 80 % of the expanded cell population comprises activated NK cells.

In natural immunity, activation of Toll-like receptors (TLR) signaling cascade has been reported to play a pivotal role in inducing immune response. In vaccine industry, various kinds of TLR ligand are being used as vaccine adjuvant. Lipopolysaccharides (LPS) are a naturally occurring TLR ligand derived from the outer membrane of gram-negative bacteria. However, the use of LPS in cell culture system may not comply with the processing requirements for the production of safe cell therapy products. CRX527 is a synthetic lipid A that belong to the aminoalkyl glucoaminide 4-phosphate (AGP) family. It has been reported that CRX527 is one of the most powerful synthetic TLR4 agonists. Advantages of using CRX527, instead of bacterial derived TLR ligands, are simple usage, non-toxic, and inexpensive.

Figure 1 shows the proportions of NK cells (CD3-CD56+ cells) produced in indicated concentrations of CRX527 treated groups.

Figure 2 shows final cell number and the proportion of NK and T cells after expand CBNK cells in the said condition.

From the experiment, it has been found that the addition of CRX527 at the concentration of 100 ng/ml during the first 7 days of the culturing procedure can increase the purity of the expanded CBNK cells.

Although this invention is described in detail, it will be understood that this invention are intended to cover any modification or solutions provided by the persons skilled in the arts. The scope of this invention is based on the claims and also cover any characteristics of the invention that may not be specifically covered in the claims, but still cause the same effects as those of this invention.

Best Mode of the Invention

As disclosed in the Detailed Descriptions of the Invention.

Claims

What is claimed is:

1. A method for large scale expansion of natural killer (NK) cells in a cell culture medium comprising the steps of:
adding said cells to a culture system comprising a growth medium supplemented with serum is selected from the group consisting of human serum and autologous plasma,
adding an effective concentration of CRX 527,
expanding said cells within said system and heating,
wherein the said effective concentration of CRX 527 in the said growth medium is in the range of 0.01 µg/ml –10 µg/ml, preferably 100 ng/ml and the ratio between the cell density and the total volume of growth medium is in the range of 1.5 to 2.
2. The method according to claim 1, wherein the cells are incubated for at least about 7 days.
3. The method according to claim 1 or 2, wherein the culture system is a pre-sterile bag.
4. The method according to claim 1 or 2, wherein the culture system is a bioreactor.
5. The method according to claim 1 or 2, wherein the culture system is a cell culture vessel.
6. A suspension of natural killer (NK) cells obtained from any of the above claims wherein at least 70 %, preferably more than 80 % of the expanded cell population comprises activated NK cells.