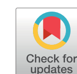
Journal of Biotechnology 287 (2018) 8–17

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Dynamic suspension culture improves *ex vivo* expansion of cytokine-induced killer cells by upregulating cell activation and glucose consumption rate



Weiwei Zhang, Haibo Cai*, Wen-Song Tan

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai, PR China

ARTICLE INFO

Key words:
CIK cells
Ex vivo expansion
Dynamic culture
Glucose metabolism
Activation state

ABSTRACT

Ex vivo expansion is an effective strategy to acquire cytokine-induced killer (CIK) cells needed for clinical trials. In this work, the effects of dynamic suspension culture, which was carried out by shake flasks on a shaker, on CIK cells were investigated by the analysis of expansion characteristics and physiological functions, with the objective to optimize the culture conditions for *ex vivo* expansion of CIK cells. The results showed that the expansion folds of total cells in dynamic cultures reached 69.36 ± 30.36 folds on day 14, which were significantly higher than those in static cultures (9.24 ± 1.12 folds, $P < 0.05$), however, the proportions of $CD3^+$ cells and $CD3^+CD56^+$ cells in both cultures were similar, leading to much higher expansion of $CD3^+$ cells and $CD3^+CD56^+$ cells in dynamic cultures. Additionally, expanded CIK cells in two cultures possessed comparable physiological functions. Notably, significantly higher percentages of $CD25^+$ cells and $CD69^+$ cells were found in dynamic cultures ($P < 0.05$). Besides, much higher glucose consumption rate of cells ($P < 0.05$) but similar $Y_{Lac/gluc}$ were observed in dynamic cultures. Further, cells in dynamic cultures had better glucose utilization efficiency. Together, these results suggested that dynamic cultures improved cell activation, then accelerated glucose consumption rate, which enhanced cell expansion and promoted glucose utilization efficiency of cells.

1. Introduction

Adoptive T cell therapy is a promising clinical treatment strategy to eliminate cancer cells and rebuild immune system (Schmidt et al., 2014). Frequently-used effector cells such as tumor infiltrating lymphocytes (TILs), cytotoxic T cells (CTLs), natural killer (NK) cells, cytokine-induced killer (CIK) cells, especially chimeric antigen receptors-T cells (CAR-T) emerging in recent years have been developed to treat various solid tumors and hematologic malignant diseases (Chacon et al., 2015; de Wilde et al., 2017; Donia et al., 2014; Gangadaran and Ahn, 2017; Jennings et al., 2014; Zhao et al., 2015).

CIK cells are generated from blood mononuclear cells *ex vivo* by the initial priming with interferon-γ (IFN-γ), anti-CD3 monoclonal antibody and interleukin-2 (IL-2), and fresh culture media were added every two or three days (Introna, 2017; Schmidt-Wolf et al., 1993, 1991). The obtained CIK cells are heterogeneous and composed of $CD3^+CD56^-$cells, $CD3^-CD56^+$cells, and $CD3^+CD56^+$cells, in which antitumor activity is mainly associated with $CD3^+CD56^+$cells (Arafar, 2014; Cappuzzello et al., 2017; Schmeel et al., 2014). It was also found that and the majority of $CD3^+CD56^+$ cell subset in CIK cultures were derived from $CD3^+CD56^-$cells, and $CD56^+$cells co-expressed CD8 antigen with negligible CD4 expression(Linn et al., 2002).

The antitumor activity of CIK cells is mainly mediated by the engagement of NKG2D and release of perforin and granzyme-containing granules(Kuci et al., 2010; Verneris et al., 2004). Due to the broad-spectrum killing tumor activity and low risk of graft-versus-host disease, CIK cells have been widely used for various cancer treatment (Gu et al., 2017; Introna et al., 2017; Zhao et al., 2015). As the more CIK cells are expanded and injected, the better they respond in patients, the dose of CIK cells used in many clinical studies has been up to 10^{10} cells per infusion (Gao et al., 2017; Hontscha et al., 2011; Introna et al., 2017; Li et al., 2012). However, the initial number of CIK cells we could get is limited; therefore, how to improve *ex vivo* expansion of CIK cells has become an hotspot in this field (Arafar, 2014; Jakel and Schmidt-Wolf, 2014; Mata-Molanes et al., 2017).

The conventional *ex vivo* expansion of CIK cells was carried out mainly in static culture with gas-permeable culture bags or T flasks (Introna et al., 2006; Jin et al., 2012; Zuliani et al., 2011). In static cultures, cells sank to the bottom of culture bags or flasks, the microenvironment surrounding cultured cells was heterogeneous because of lacking mixing (Curcio et al., 2012; Sadeghi et al., 2011). Nevertheless, the environmental inhomogeneity could be overcome by the use of

* Corresponding author at: State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, P. O. Box 309#, Shanghai 200237, PR China.

E-mail address: caihaibo@ecust.edu.cn (H. Cai).

https://doi.org/10.1016/j.jbiotec.2018.09.010
Received 26 January 2018; Received in revised form 17 May 2018; Accepted 26 September 2018
Available online 29 September 2018
0168-1656/ © 2018 Elsevier B.V. All rights reserved.

dynamic suspension culture in bioreactors. In bioreactor-based dynamic suspension cultures, process parameters such as temperature, pH, gases (*e.g.*, O_2 and CO_2) and nutrient levels, can be better monitored (Kropp et al., 2017). Hence, various devices including stirred bioreactors, wave bioreactors, etc. have been developed for *ex vivo* expansion of therapeutic cells, such as CTLs, NK cells and TILs. For instance, Bohnenkamp et al. (2002) demonstrated that more $CD3^+$ T cells were expanded in a stirred bioreactor than in T-flasks during the *ex vivo* expansion of T cells. In line with this, Spanholtz et al. (2011) used wave bioreactors to expand NK cells *ex vivo*, leading to a higher expansion folds of $CD3^-CD56^+$cells than that of static bag cultures. Also, Donia et al. (2014) illustrated a 4-times higher expansion fold of TILs in wave bioreactors than in static bag cultures. However, different or even contrary results were set out in some other researches. For example, Sutlu et al. (2010) indicated a lower expansion folds of $CD3^-CD56^+$cells in wave bioreactor cultures compared with T-flask cultures in the *ex vivo* expansion of NK cells. Moreover, Somerville et al. (2012) demonstrated a comparable expansion of TILs in wave bioreactors and static culture bags. A possible explanation of these conflict results could be found in the expansion setup used in these studies with different control of process parameters including cell density, media exchange, nutrient levels, etc. It had been certified that cell density played an important role in the activation, *ex vivo* expansion and survival of immune effector cells. When cell seeding density was lower than 1×10^5 cells/ml, T cells could not be activated adequately; only when cell seeding density was over 1×10^5 cells/ml, T cells would be activated; for survival and further expansion, T cells had to be maintained over the density of 1×10^5 cells/ml or even higher (Ma et al., 2010). In addition, cell density was often readjusted to $0.3 \sim 2 \times 10^6$ cells/ml by fresh medium supplement as required during the *ex vivo* expansion of TILs, NK cells or CAR-T cells in some studies (Malone et al., 2001; Rujkijyanont et al., 2013; Wang et al., 2012; Zuliani et al., 2011). Hence, more attention should be paid to the cell density of seeding and maintaining in the *ex vivo* culture of immune effector cells. Except cell density, appropriate levels of glucose and sub-toxic levels of lactate also had important effects on cell productivity (Roh et al., 2016; Weegman et al., 2013). Glucose is an important carbon source necessary for the growth, proliferation, and functions of T cells (van der Windt and Pearce, (2012)). Maintaining different glucose level resulted in different cell expansion. Somerville et al. (2012) kept the glucose levels around 8.33 mmol/L, demonstrating a 1281 ± 100 expansion fold of TILs in wave bioreactors, while Sadeghi et al. (2011) maintained glucose levels within the range of $8.33 \sim 11.11$ mmol/L resulting in a 120 ± 28-fold expansion of TILs in wave bioreactors. Therefore, process optimization should be conducted to improve the *ex vivo* expansion of immune effector cells using various methods such as kinetic analysis of glucose consumption and lactate accumulation (Zhang et al., 2015a). Up to now, however, in most cases of CIK cells manufacturing, far too little attention has been paid to these critical processes and kinetic parameters.

Besides, during *ex vivo* expansion, only lymphocytes which were activated enough could enter into cell cycle and undergo proliferation (Appleman and Boussiotis, 2003; Schwartz, 2003), activated lymphocytes distinguished themselves from naïve cells by expressing certain molecules including CD25, CD69, etc. on their cell surfaces (Shipkova and Wieland, 2012). CD69 was the earliest inducible cell surface glycoprotein and functioned as a signal transmitting receptor, which involved in the expression of late activation markers and cell proliferation, the expression of CD69 could reliably predict the anti-CD3-induced proliferative response of lymphocytes(Prince and Lape-Nixon, 1997). CD25 was the alpha chain of IL-2 receptor (IL-2RA), together with beta chain and the common gamma chain, constituting the high-affinity IL-2 receptor, while IL-2 was one of the most important cytokine in CIK *ex vivo* expansion, so the expression of CD25 was also important to cell proliferation(Sim and Radvanyi, 2014). And it had been suggested that these activation antigens were upregulated on proliferating lymphocytes and the activation makers expression paralleled with lymphocytes proliferation (Maino et al., 1995; Shipkova and Wieland, 2012), so the activation state of cultured cells during may need to be investigated during the *ex vivo* expansion of CIK cells.

In the present work, a dynamic suspension culture process for *ex vivo* expansion of CIK cells was adopted, with the objective to develop optimal cell culture process. Particularly, cell growth, cell population and physiological function of expanded cells in dynamic suspension culture were characterized. Specifically, and the activation state of cells in two cultures and the changes of glucose concentration, lactate concentration and pH during culture process were measured. Furthermore, kinetic parameters including cell growth rate, glucose consumption rate, lactate production rate of cells, glucose utilization efficiency were further investigated to better understand the effects of dynamic cultures on CIK cell expansion. The findings may lay the foundation for the optimization of *ex vivo* CIK cells expansion in bioreactors, eventually favoring their therapeutic applications.

2. Materials and methods

2.1. Isolation of CBMNCs and ex vivo expansion of CIK cells

The experiments conducted in this study were approved by the Science Ethics committee of the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology and were in accordance with the guidelines for cellular products research and preparation, China (2016). CIK cells were generated from cord blood mononuclear cells (CBMNCs) of full-term healthy delivery with informed consent. Low-density mononuclear cells were enriched using density gradient centrifugation on Ficoll/Histopaque (density: 1.077 *g*/ml, GE healthcare, USA). CBMNCs were resuspended in growth medium and prepared for use.

CBMNCs were seeded at 2×10^6 cells/ml into 125 ml shake flasks (Corning, USA) in duplicate, one was for static culture, and the other was for dynamic culture. Culture medium was RPMI1640 medium including 10% Fetal Bovine Serum (FBS) (Biosun, China), 1000 IU/ml IFN-γ (Peprotech, USA), 50 ng/ml anti-CD3 antibody (Ebioscience, USA), 100 IU/ml IL-1α (Peprotech, USA), 500 IU/ml IL-2 (Peprotech, USA) and 500 ng/ml PHA (Sigma-Aldrich, Germany). Fresh RPMI1640 medium with 10% FBS and 500 IU/ml IL-2 was added daily to maintain the viable cell density at 2×10^6 cells/ml. For static and dynamic cultures, shake flasks were placed on a shaker at a speed of 0 or 130 rpm, and the shaker were placed at 37 °C in a humidified incubator with 5% CO_2. Culture supernatants in static and dynamic cultures were mixed sufficiently before sampling. pH was measured using a pH meter (Mettler-Toledo, USA). Cell numbers were counted every day. The kinetics of cell growth was calculated according to the following equation.

Specific growth rate:

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \tag{1}$$

Wherein μ was the specific growth rate of cells, N_1 was the number of cells at the time point of t_1, N_2 was the number of cells at the time point of t_2.

2.2. Determination of the proportions of $CD3^+$cells, $CD3^+CD56^+$ cells, $CD3^+CD8^+$ cells and $CD3^+CD4^+$ cells

A total of 1×10^6 cells was collected, rinsed with phosphate- buffered saline (PBS) and resuspended in 50 µl of PBS, cells were stained with FITC-conjugated anti- human CD3 antibody (BD, USA) and incubated for 30 min at 4 °C in dark. For the detection of $CD3^+CD56^+$ cells, $CD3^+CD8^+$ cells and $CD3^+CD4^+$ cells, cells pretreated with the CD3 antibody were further stained with PE-conjugated anti-human CD56antibody (BD, USA), Percp-cy5.5-conjugated anti-human CD8

Download English Version:

https://daneshyari.com/en/article/11016665

Download Persian Version:

https://daneshyari.com/article/11016665

Daneshyari.com