Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy ISCT®

Large-scale expansion of Vγ9Vδ2 T cells with engineered K562 feeder cells in G-Rex vessels and their use as chimeric antigen receptor–modified effector cells

LIN XIAO[1,*], CAN CHEN[2,*], ZHENDONG LI[1], SUMIN ZHU[1], JOHAN CK TAY[1], XI ZHANG[1], SHIJUN ZHA[1], JIEMING ZENG[3], WEE KIAT TAN[2], XIN LIU[4], WEE JOO CHNG[4,5,6] & SHU WANG[1,3]

[1]Department of Biological Sciences, National University of Singapore, Singapore, [2]Tessa Therapeutics, Pte Ltd., Singapore, [3]Institute of Bioengineering and Nanotechnology, Singapore, [4]Department of Haematology-Oncology, National University Cancer Institute Singapore, National University Health System, Singapore, [5]Cancer Science Institute of Singapore, National University of Singapore, Singapore, and [6]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Abstract

Vγ9Vδ2 T cells are a minor subset of lymphocytes in the peripheral blood that has been extensively investigated for their tolerability, safety and anticancer efficacy. A hindrance to the broad application of these cells for adoptive cellular immunotherapy has been attaining clinically appropriate numbers of Vγ9Vδ2 T cells. Furthermore, Vγ9Vδ2 T cells exist at low frequencies among cancer patients. We, therefore, sought to conceive an economical method that allows for a quick and robust large-scale expansion of Vγ9Vδ2 T cells. A two-step protocol was developed, in which peripheral blood mononuclear cells (PBMCs) from healthy donors or cancer patients were activated with Zometa and interleukin (IL)-2, followed by co-culturing with gamma-irradiated, CD64-, CD86- and CD137L-expressing K562 artificial antigen-presenting cells (aAPCs) in the presence of the anti-CD3 antibody OKT3. We optimized the co-culture ratio of K562 aAPCs to immune cells, and migrated this method to a G-Rex cell growth platform to derive clinically relevant cell numbers in a Good Manufacturing Practice (GMP)-compliant manner. We further include a depletion step to selectively remove αβ T lymphocytes. The method exhibited high expansion folds and a specific enrichment of Vγ9Vδ2 T cells. Expanded Vγ9Vδ2 T cells displayed an effector memory phenotype with a concomitant down-regulated expression of inhibitory immune checkpoint receptors. Finally, we ascertained the cytotoxic activity of these expanded cells by using nonmodified and chimeric antigen receptor (CAR)–engrafted Vγ9Vδ2 T cells against a panel of solid tumor cells. Overall, we report an efficient approach to generate highly functional Vγ9Vδ2 T cells in massive numbers suitable for clinical application in an allogeneic setting.

Key Words: *antibody-dependent cell-mediated cytotoxicity, artificial antigen-presenting cells, chimeric antigen receptor, gamma delta T cells*

Introduction

Vγ9Vδ2 T cells are a subset of γδ T cells that make up approximately 0.5–5% of peripheral blood T cells [1]. Unlike classical αβ T cells, Vγ9Vδ2 T cells express Vγ9 and Vδ2 of T-cell receptor (TCR) chains that recognize and interact with antigens in a major histocompatibility complex (MHC)–independent fashion. γδ TCRs can be activated by a set of tumor-associated antigens, including phosphoantigens that are produced during metabolic dysregulation in tumor cells, lipids presented by CD1 family members and cell stress markers [2–4]. Like innate natural killer (NK) lymphocytes, γδ T cells also express the NKG2D receptor and killer-cell immunoglobulin-like receptors (KIRs) that can play either co-stimulatory or inhibitory roles. As such, γδ T cells are considered to play important roles in immune surveillance against tumors.

*These two authors contributed equally to the work.

Correspondence: **Shu Wang**, PhD, Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543, Singapore. E-mail: dbsws@nus.edu.sg

(Received 21 September 2017; accepted 29 December 2017)

ISSN 1465-3249
https://doi.org/10.1016/j.jcyt.2017.12.014

Importantly for cancer treatment, γδ T cells are capable of infiltrating a range of human solid tumors, including renal, bladder, ovarian, colorectal, breast and nasopharyngeal carcinomas, to interact with and kill cancer cells [5].

Within the larger peripheral blood mononuclear cell (PBMC) population, the Vγ9Vδ2 T-cell subset can be specifically expanded with the treatment of phosphoantigens such as isopentenyl pyrophosphate (IPP) or its synthetic analogue of bromohydrin pyrophosphate (BrHPP). Zoledronate or zoledronic acid (Zometa), a Food and Drug Administration (FDA)-approved and commercially available bisphosphonate drug, which has been used to treat patients with postmenopausal osteoporosis, has also been used to artificially raise the intracellular levels of IPP by inhibiting farnesyl pyrophosphate synthase (FPPS), an enzyme acting downstream of IPP in the mevalonate pathway [2–4]. Initial Zometa/Vγ9Vδ2 T-cell immunotherapy used an *in vivo* expansion approach through direct administration of Zometa into patients. Vγ9Vδ2 T cells have also been tested for adoptive cancer immunotherapy, a fast-developing field that involves the isolation of immune cells, *ex vivo* cell expansion and re-infusion of the expanded lymphocytes into patients to treat cancer. The expansion of Vγ9Vδ2 T cells *ex vivo* with Zometa together with cytokines allows for the reproducible generation of large numbers of effector cells, the optimization and control of cell population purity and the maintenance and augmentation of cell function and survival. In several early-phase clinical trials of adoptive immunotherapy for cancer, treatments with Zometa-expanded peripheral blood Vγ9Vδ2 T cells are very well tolerated and have yielded some encouraging positive clinical outcomes [5–10]. A meta-analysis of gene expression signatures of a panel of 39 tumor types has identified the presence of intratumoral γδ T cells as the most significant indicator for favorable prognosis that is positively correlated with patient survival [11].

Advances in adoptive immunotherapy with Vγ9Vδ2 T cells can be improved significantly through developing methods for large-scale, clinically relevant cell expansion over a short period of time. There have been major advances in this regard over the last several years [12–16]. Artificial antigen-presenting cells (aAPCs) generated through genetic engineering of human erythroleukemia cell line K562 have been used to support robust *ex vivo* expansion of several types of immune cells [17,18]. Deniger *et al.* [19] have reported the use of K562-derived aAPCs that express membrane-*bound IL* (mIL)-15, CD86 and CD137L for polyclonal expansions of heterogeneous gamma-delta T subsets after the isolation of γδ T cells from PBMCs. A recent publication reports the use of K562-aAPCs expressing CD83, CD137L and CD32 for the expansion of Vγ9Vδ2 T cells freshly isolated from PBMCs [20]. In this respect, we have developed another K562 line expressing CD64, CD86 and CD137L and recently reported the use of the K562-aAPCs for the co-expansion of cytokine-induced killer cells and Vγ9Vδ2 T cells [21].

In the current study, we focused on the use of K562 aAPCs in G-Rex cell culture vessels for Vγ9Vδ2 T-cell expansion. A two-step protocol was developed in which PBMCs were first activated with Zometa and interleukin (IL)-2 for 7 days and the activated cells were then numerically expanded by co-culturing with K562-aAPCs, anti-human CD3 monoclonal antibody OKT3, Zometa and IL-2 in G-Rex cell culture vessels for 10 days. The Good Manufacturing Practice (GMP)-compliant G-Rex vessels contain a gas-permeable membrane at the base and are unique in supporting large media volumes without compromising gas exchange [22–30]. Our results demonstrate that the new method enables the generation, from relatively small amounts of peripheral blood cells, of large clinically relevant quantities of functional Vγ9Vδ2 T cells in 17 days and the expanded Vγ9Vδ2 T cells are suitable for use as either unmodified or chimeric antigen receptor (CAR)–engrafted effector cells.

Results

Ex vivo *expansion of Vγ9Vδ2 T cells with a two-step protocol*

Vγ9Vδ2 T cells are commonly cultured and expanded via stimulation with Zometa. In our two-step protocol (Figure 1A), PBMCs were first treated with Zometa in combination with IL-2 in a serum-supplemented growth medium for 7 days to enrich Vγ9Vδ2 T cells. Although the total number of cells did not increase significantly during this 7-day period, the Vγ9Vδ2 T-cell population could increase from an initial 1–5% in the original untreated PBMCs (day 0) to 70–80% after Zometa treatment for 7 days. In the second step, Zometa-treated cells were mixed with irradiated K562-aAPCs stably expressing three genes that encode the high affinity Fc receptor CD64 and co-stimulatory molecule ligands CD86 and CD137L (4-1BBL) [21] (Supplementary Figure S1). This co-culture was performed for 10 days with the serum-supplemented medium containing OKT3, Zometa and IL-2.

In trying to define an optimized K562-aAPC co-culture condition, we tested the effects of different cell ratios of immune cells to K562-aAPCs in the second step, from 1:0 to 1:200, on Vγ9Vδ2 T-cell expansion in cell culture flasks. While the cells that were continually treated with Zometa for 10 days, without adding K562-aAPCs (ratio = 1:0), were expanded on an average of 72-fold, co-culturing with K562-aAPCs provided expansion folds from 376-fold at 1:2 ratio to