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Generation of antigen-specific cytotoxic T lymphocytes with activated B cells

SUN OK YUN[1], HEE YOUNG SHIN[1], CHANG-YUIL KANG[2] & HYOUNG JIN KANG[1]

[1]*Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea, and* [2]*Laboratory of Immunology, College of Pharmacy, Seoul National University, Seoul, Republic of Korea*

Abstract

Background aims. Dendritic cells are well known as the most potent antigen-presenting cells. Nonetheless, their use in immunotherapy has been limited by the time-consuming and laborious steps involved in their generation *in vitro*. Therefore, much attention has been placed on alternative antigen-presenting cells that are relatively more convenient to manipulate. *Methods.* In this study, the efficacy of B cells as antigen-presenting cells, compared with dendritic cells, in the induction of cytotoxic T lymphocytes against cytomegalovirus-specific antigens was evaluated. B cells were isolated from the peripheral blood mononuclear cells of healthy individuals, loaded with α-galactosylceramide for activation, and nucleofected with cytomegalovirus-antigen coding plasmid DNA. Antigen-nucleofected B cells or dendritic cells were cocultured with T cells for 14 days *in vitro*. *Results.* The proliferation of cytotoxic T lymphocytes induced by B cells was similar to that of those induced by dendritic cells. Additionally, the immunogenicity of both sets of cytotoxic T lymphocytes was similar not only in interferon-γ enzyme-linked immunospot assays but also in cytotoxicity assays. *Discussion.* These observations suggest that α-galactosylceramide-loaded B cells could be used as antigen-presenting cells as an alternative to dendritic cells. Using B cells has several benefits, including cost-effectiveness and being both less time-consuming and less labor-intensive.

Key Words: *B cells, DNA nucleofection, α-galactosylceramide (α-GalCer), cytotoxic T lymphocytes (CTLs)*

Introduction

Post-transplant patients have significant morbidity and mortality due to infections, particularly those caused by viruses [1–3]. Antiviral drugs, which are the standard therapy, are frequently ineffective, toxic and can induce drug resistance [4,5]. In contrast, the adoptive transfer of antigen-specific cytotoxic T-lymphocyte cells (CTLs) as treatment after transplantation has shown clinical efficacy without significant toxicity and may be an attractive alternative strategy for the prevention or treatment of viral infections after hemopoietic stem cell transplantation [6–8].

To induce viral or tumor antigen–specific CTLs, professional antigen-presenting cells (APCs) capable of activating T cells are required. Although dendritic cells (DCs), B cells and macrophages are well known as professional APCs, DCs are the most popular in many key stages, such as antigen processing, antigen presentation and migration [9–11]. However, even though they are the most potent professional APCs, there are disadvantages to using DCs, including high cost and a time-consuming and laborious method of generating them from monocytes *ex vivo* [12,13].

In several previous studies, B cells were used as an alternative source of potent APCs because of their abundance in blood and lymphoid organs as well as their ease of preparation [13–15]. Although it has been suggested that B cells may be used as APCs in cell-based immunotherapy, they have been considered inadequate because of their low immunogenicity and induction of tolerance [13,16,17]. However, in previous studies looking at the activation of the immune response by B cells, it had been suggested that they are able to induce activation with the invariant natural killer T (NKT) cell ligand, α-galactosylceramide (α-GalCer) [14,15], a well-known immune adjuvant and NKT cell stimulator [18,19]. α-GalCer loaded on B cells can activate NKT cells; subsequently, α-GalCer-loaded B cells could, in turn, be activated by activated NKT cells because of the upregulation of costimulatory molecules [15,20]. Moreover, previous studies have found that α-GalCer-loaded B cells are long-lasting APCs *in vivo* as well as effective APCs in the sustainment of a long-lasting adoptive immune response [14].

Correspondence: **Hyoung Jin Kang**, Ph.D., M.D., Division of Hematology/Oncology, Department of Pediatrics, Cancer Research Institute, Seoul National University College of Medicine, 101 Daehangno, Chongno-gu, Seoul 110-744, Republic of Korea. E-mail: kanghj@snu.ac.kr

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In this study, we tested whether α-GalCer-loaded B cells could induce antigen-specific CTLs *in vitro* against the cytomegalovirus (CMV) antigens pp65 and IE1, similar to DCs. We chose the nucleofection of full-length antigen-encoding plasmid DNA as the antigen delivery method. Nucleofection is a rapid and efficient method for transferring DNA into the nucleus of APCs, resulting in high expression of the transgene without changing the phenotypic characteristics of the cells [21–24]. Here we suggest an effective alternative method to allow the rapid generation of antigen-specific CTLs for immunotherapeutic purposes. To demonstrate this, we induced CMV antigen-specific CTLs with α-GalCer-loaded B cells to investigate the cytotoxic activity of the CTLs. Our studies showed that antigen-specific CTLs with activated B cells have as powerful a cytotoxicity as CTLs with DCs, suggesting a new possibility of using *ex vivo* CTLs induced by activated B cells.

Methods

Donors and peripheral blood mononuclear cell preparation

Blood samples were collected from healthy volunteers, after informed consent had been obtained. Peripheral blood mononuclear cells (PBMCs) from healthy individuals were obtained by leukapheresis using Ficoll-Paque PLUS (GE Healthcare) density gradient centrifugation. This study was approved by the Institutional Review Board for Human Research of Seoul National University (IRB No. C-1207-087-418).

Generation of DCs

DCs were generated as previously described [25]. In brief, PBMCs were seeded in RPMI 1640 medium (Welgene) for 1 h, after which non-adherent cells were removed. The adherent cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen), 800 U/mL granulocyte macrophage colony-stimulating factor (ProSpec), 1000 U/mL interleukin (IL)-4 (ProSpec), and 2 mmol/L Glutamax-I (Gibco) for 5 days. Media were replenished on day 3 of culture. On day 5, immature DCs were matured with a cytokine cocktail containing 100 ng IL-6 (ProSpec), 10 ng IL-1β (ProSpec), 10 ng tumor necrosis factor-α (ProSpec) and 1 μg prostaglandin E2 (Sigma) for 48 h.

B-cell isolation and activation

B cells were isolated from PBMCs using the B-cell Isolation Kit II (Miltenyi Biotec) following the manufacturer's instructions. Magnetic-activated cell-sorting purification was carried out through negative selection. The purity of the preparation was >94% in all experiments. α-GalCer was purchased from Funakoshi Co. Isolated B cells were cocultured with 1 μg/mL α-GalCer for 18–20 h in complete culture medium.

Plasmid

The plasmid pCK-IRES was obtained from ViroMed. To construct pCK-IE1-IRES-pp65, subcloning was performed.

Nucleofection

DCs and B cells were nucleofected using the AMAXA Human B cell Nucleofector Kit (Lonza). 1–5 × 10^6 DCs or B cells were nucleofected with 1–5 μg plasmid DNA, according to the manufacturer's instructions. Nucleofected DCs and B cells were immediately resuspended in their respective culture medium and cultured for 12–18 h.

CTL generation

CD19-negative PBMCs were used as responder cells (R). Nucleofected DCs and B cells were used as stimulator cells (S). For the stimulation, S cells and R cells were cocultured at an S:R ratio of 1:10 in CTL medium (RPMI 1640 medium supplemented with 45% Click's medium [Irvine Scientific]; 2 mmol/L Glutamax-I and 10% FBS). On day 7, CTLs were harvested and restimulated at an S:R ratio of 1:10 in CTL medium with 50 ng/mL IL-15 (ProSpec). On day 14, CTLs were harvested and assayed.

Flow cytometry analysis

For cell surface staining, cells were processed following standard procedure. We stained the CTLs with monoclonal antibodies to CD3, CD4, CD8, Vα24Jα18, CD45RA and CD62L (BD Pharmingen). Cells were washed once with Dulbecco's phosphate-buffered saline (Lonza) containing 2% FBS, and the cells were incubated for 15 min at 4°C with antibody. After incubation, the cells were washed twice with identical buffer. FACS analysis was performed on FACSCanto II (BD Biosciences).

ELISpot assay

Enzyme-linked immunospot (ELISpot) analysis was performed as described [26]. The viral-specific activity of responder cells was measured after stimulation of stimulator cells with pepmixes spanning pp65 and IE1 (CMV). All pepmixes, which are overlapping peptide libraries (15-mers with 11 amino acids overlapping) were purchased from JPT Technologies. Briefly, pepmix-pulsed autologous PBMCs as stimulator cells and CTLs as responder cells were plated

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