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# Generation of Antigen-Specific Cytotoxic T Lymphocytes with Peripheral B Cells Activated By α-Galactosylceramide

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## Abstract

Dendritic cells (DCs) are well known as the most potent professional antigen presenting cells (APCs). Nonetheless, the use of these cells in immunotherapy has been limited due to the time consuming and laborious steps required to generate DCs from monocytes *in vitro*. Therefore, alternative APCs has drawn much attention because of their relative convenience in manipulation.

In this study, the efficacy of B cells as APCs, as compared to DCs, in induction of cytotoxic T lymphocytes (CTLs) against cytomegalovirus (CMV)-specific antigens was evaluated. B cells were isolated by depletion of peripheral blood mononuclear cell (PBMCs) from healthy individuals with MACS system, loaded with α-galactosylceramide (α-GalCer) for inducing B cell activation, and nucleofected with CMV-antigen coding plasmid DNA, pCK-pp65-IRES-IE1. As other APCs, monocyte-derived DCs were induced with various cytokines (GM-CSF, IL-4, IL-1b, TNF-a), for 6 days and nucleofected with the same plasmid DNA. Ag-nucleofected B cells or DCs were cocultured with T cells for 14 days *in vitro*. The cells were harvested and subsequently immunoassayed.

Proliferation of cells was more expanded by about 25~32% in CMV-CTLs induced by DCs compared to of B cells, but there was no significant difference in immunogenicity between CMV-CTLs induced with B cells and DCs. Compared to CMV-CTLs induced by DCs, the CTLs induced by α-GalCer-loaded B cells

induced similar cytotoxicity against CMV antigen (Ag) *in vitro*. The CMV-CTLs by α-GalCer-loaded B cells recognized CMV antigen pp65 (median 88 SFC/105) and IE-1 (median 86 SFC/105) in donor 1, and CMV antigen pp65 (median 31 SFC/105) and IE-1 (median 37 SFC/105) in donor 2. Similarly, the CMV-CTLs by DCs recognized CMV antigen pp65 (median 133 SFC/105) and IE-1 (median 32 SFC/105) in donor 1, and CMV antigen pp65 (median 37 SFC/105) and IE-1 (median 43 SFC/105) in donor 2.

Immunogenicities of both CTLs were similar not only on IFN-γ ELISPOT (Enzyme-linked immunospot) assay but also on cytotoxicity assay. The CMV-CTLs by α-GalCer-loaded B cells have killing activity against CMV antigen pp65 (100%, at E:T ratio 10:1) and IE1 (85%, at E:T ratio 10:1) in donor 1, and CMV antigen pp65 (69%, at E:T ratio 10:1) and IE1 (27%, at E:T ratio 10:1) in donor 2. Also, the CMV-CTLs by DCs show killing activity against CMV antigen pp65 (100%, at E:T ratio 10:1) and IE1 (42%, at E:T ratio 10:1) in donor 1, and CMV antigen pp65 (88%, at E:T ratio 10:1) and IE1 (64%, at E:T ratio 10:1) in donor 2.

These observations suggest that α-GalCer-loaded B cells could be used in general as APCs instead of DCs. Using the B cells as APCs have several benefits such as cost-effectiveness, less time-consuming, and less laborious compared to when DCs are used. Furthermore, nucleofection technique might be useful in delivering antigen-coding DNA, not only for virus antigens but also for tumor antigens, directly into the nucleus. Our results demonstrate that α-GalCer-loaded B cells could be potent APCs in generating antigen-specific CTLs for cellular vaccines and adoptive immunotherapy.

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**Disclosures**

No relevant conflicts of interest to declare.

# Author notes

*Asterisk with author names denotes non-ASH members.