Gamma Irradiated Human Platelet Lysate as a Serum Substitute for *Ex Vivo* Expansion of Immune Cells

Yee-Hsien Lin[1], Wen-Ting Yang[1], Fang-Yu Liang[1], Rong-Jeng Tseng[1,2], Min-Chang Huang[1,2]
[1]AventaCell BioMedical Corp. Ltd., New Taipei City, Taiwan [2]AventaCell BioMedical Corp., Atlanta, Georgia, USA
Author Email: rjtseng@atcbiomed.com

Introduction

Adoptive immunotherapy is emerged as one of the leading treatments in cancer. The clinical applications require ex vivo expansion of lymphocytes to therapeutic doses in a timely manner under strict cGMP compliance. Culture medium is one important determinant for successful cell expansion. Many efficient expansion strategies incorporate serum or plasma as a supplement to provide cells with a richer milieu to grow in. However, autologous serum/plasma has supply limitations for meeting the expected commercial demand for immunotherapies. Moreover, pooled human serum or animal-derived serum bring the inherent concern about risk of infection transmission.

Human platelet lysate (hPL) is well recognized as an efficient xenogenic-free alternative to FBS for manufacturing cells and cellular products. To address the risk management for pathogen transmission, we have assessed the feasibility of gamma irradiation as a pathogen reduction treatment for hPL and validated its efficacy for virus inactivation. Our gamma irradiation process demonstrated low impact on hPL potency and long-term stability.

Objectives

In this study, we evaluated the potential use of gamma-irradiated human platelet lysate on immune cell expansion.

Methods

Expansion of NK cells

- Isolated peripheral blood mononuclear cells (PBMC) were cultured in anti-CD16 Ab coated flasks and stimulated with IL-2 (1000 U/mL) for 3 days.
- Then the cultured cells were transferred to new flasks and supplied with fresh medium (UltraKURE) and IL-2 in the presence of various supplements. Upon each media exchange, the numbers of total nucleated cells were counted.

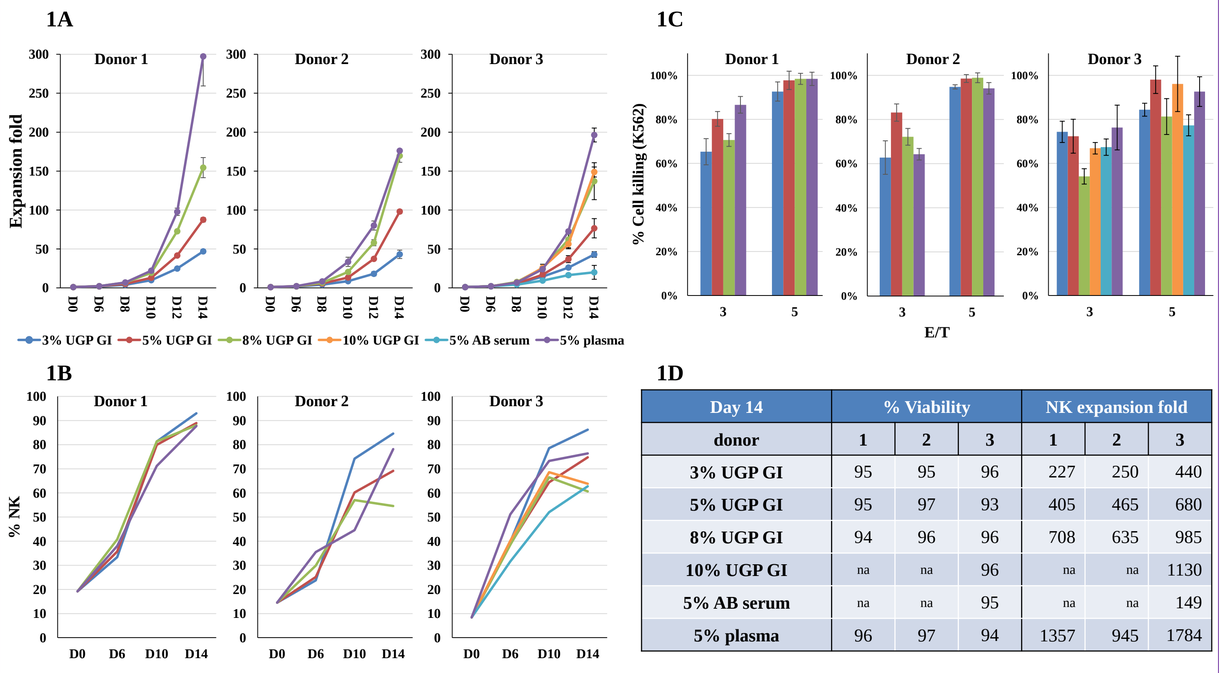
Expansion of T cells

- At day 0, PBMCs were stimulated by CD3/CD28 beads and IL-2 (100 U/mL) in medium (WK552S) with supplements.
- At day 10, cells were re-stimulated by CD3/CD28 beads.

Evaluation of expansion performance

At the end of cell expansion, expression of cell surface markers, CD3 and CD56, was analyzed via flow cytometry. The cytotoxicity of expanded NK cells against the target cells (K562) was accessed by a fluorescence based assay.

Results



Day 14	% Viability			NK expansion fold		
donor	1	2	3	1	2	3
3% UGP GI	95	95	96	227	250	440
5% UGP GI	95	97	93	405	465	680
8% UGP GI	94	96	96	708	635	985
10% UGP GI	na	na	96	na	na	1130
5% AB serum	na	na	95	na	na	149
5% plasma	96	97	94	1357	945	1784

Figure 1. NK cell expanded from PBMC in medium (UltraKURE) supplemented with gamma-irradiated human platelet lysate (UGP GI) or autologous plasma or AB serum. (A) The total numbers of cells were counted at the indicated days and calculated the total cell fold expansion. After 14 days in culture, expended cells were analyzed for (B) the percentage of CD3-/CD56+ expressing cells (C) the cytotoxicity of NK cells against K562 cells at indicated effector:traget ratios (D) the percentage of live cells and the specific expansion fold of CD3-/CD56+ NK cells.

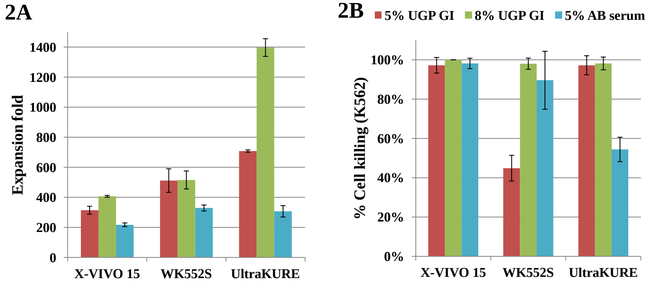


Figure 2. Expansion of NK cell line (NK-92) in various cell culture media with IL-2 (200U/mL) and different supplements.

After 12 days in culture, expended cells were analyzed for (A) The expansion fold of NK cells (B) Cytotoxicity of NK cells against K562 cells at 5:1 (effector : target) ratio.

Medium used:
X-VIVO 15 (Lonza); WK552S (Takara); UltraKURE (AventaCell)

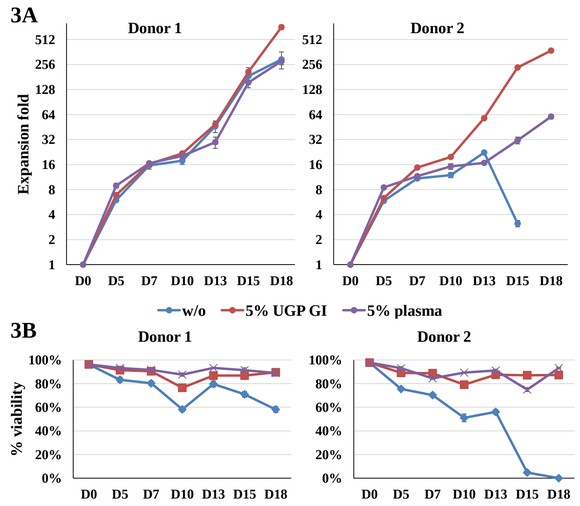


Figure 3. T cell expanded from PBMC in medium (WK552S) supplemented with gamma-irradiated human platelet lysate or autologous plasma. The total numbers of cells were counted at the indicated days for calculating (A) the total cell fold expansion and (B) the percentage of live cells.

Conclusion

NK cell expansion

- The potency of UGP GI and autologous plasma were comparable while higher than AB serum. The expansion folds were more consistant among donors with UGP GI supplemented medium.
- The cytotoxicity of NK cells against K562 cells was high and comparable for both UGP GI and plasma.
- UGP GI promoted higher NK-92 expansion than AB serum while retained high cytotoxicity.

T cell expansion

- UGP GI supplemented medium showed higher yields than the expansion obtained with autologous plasma.

The results suggest that gamma-irradiated human platelet lysate (UGP GI) is a viable and valuable alternative cell culture supplement for ex vivo expansion of immune cells.

www.atcbiomed.com