SOP: D03.14.23 ADENOVIRUS TRANSDUCTION OF B95-8+ LCL FOR CTL STIMULATION

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1. Purpose

1.1. Epstein-Barr Virus-transformed B cell lines for use as stimulators for cytotoxic T-lymphocytes may be derived from peripheral blood of the stem cell donor or the patient.

1.2. B cells are infected by a well-characterized laboratory strain of EBV to generate a lymphoblastoid cell line (LCL).

1.3. Antigen can be introduced into the LCL by transduction with adenoviruses.

2. Scope

2.1. This procedure is to be followed by GMP staff to transduce lymphoblastoid cells to generate clinical grade cytotoxic T cells.

3. Definitions and Abbreviations

3.1.	CTL	Cytotoxic T- lymphocytes
3.2.	EBV	Epstein-Barr Virus
3.3.	PBMNC	Peripheral blood mononuclear cells
3.4.	LCL	Lymphoblastoid Cell Line
3.5.	B95-8	A laboratory strain of EBV
3.6.	HI	Heat Inactivated
3.7.	LCL medium	RPMI 1640 containing 10% HI FCS and 2mM L-glutamine and 100 μM acyclovir
3.8.	CTL medium	RPMI 1640 45%, Clicks' 45% containing 10% HI FCS and 2mM L-glutamine

4. Materials and Equipment:

NOTE: All materials in contact with cells must be sterile, pyrogen-free, stored and used according to the manufacturer's directions unless stored otherwise. Equivalent materials and equipment may be used but all changes must be recorded in the appropriate worksheets.

4.1. Specimen

4.1.1. B95-8+LCL generated according to SOP D03.01.

4.2. Materials

4.2.1.	RPMI 1640	Hyclone
4.2.2.	Dulbecco's PBS	Invitrogen
4.2.3.	Fetal Calf Serum	HyClone
4.2.4.	L-glutamine(200 mM)	Invitrogen
4.2.5.	Acyclovir	Wellcome
4.2.6.	24-well plate	Costar
4.2.7.	Clinical Grade Adenovirus	Vector Production Facility
4.2.8.	Centrifuge tubes	Falcon
4.2.9.	Serological Pipets	Falcon

4.3. Equipment

4.3.1. Biological safety cabinet, certified

4.3.2. Centrifuge

4.3.3. Incubator

4.3.4. Hemacytometer

4.3.5. Microscope

5. Procedure:

5.1. Adenovirus transduction of B95-8+ LCL line

5.1.1. Harvest all cells and spin at 400G for 5mins and resuspend in a known volume

5.1.2. Perform live cell count using hemacytometer according to SOP E03.11

5.1.3. Aliquot up to $10x10^6$ LCL per 15 ml centrifuge tube or up to 40x10e6 in a 50ml tube.

5.1.4. Centrifuge @ 400 g at ambient temperature for 5 minutes to pellet cells.

5.1.5. Aspirate supernatant.

5.1.6. Finger flick tube to disperse cell pellet.

5.1.7. Transfer adenovirus vector into the cells and pipette to mix. The volume to be added depends on the vector being used and will be decided by the protocol PI.

5.1.7.1. For Ad-LMP1-I-LMP2 transduction, use a multiplicity of infection (MOI) of 15,000 viral particles (vp) per cell in 500 μl; e.g., If titer of Adenovirus = 10^{12} vp/ml, add 15μl virus/ 1x10^6 cells.

5.1.7.2. For Ad-CMVpp65 transduction, use an MOI of 100 vp per cell; e.g., If titer of Adenovirus is 1 x 10^{12} vp/ml, dilute 1:100 and add 10μl of the diluted virus/ 1x10^6 cells.

5.1.8. Loosen cap on centrifuge tube slightly for CO_2 exchange.

5.1.9. Incubate for approximately 90 minutes at 37°C/5% CO_2.

5.1.10. After 90 minutes, resuspend cells in complete culture medium to a concentration of 5x10^5 cells/ml.

5.1.11. Add 2 mls of cell suspension to each well of a 24-well plate.

5.1.12. Incubate for 2 days in 37°C, 5% CO_2 incubator.

5.1.13. LCL are now ready for use as stimulators.

5.2. Preparation of Transduced LCL for CTL stimulation

5.2.1. Perform cell count using hemacytometer according to standard operating procedure and calculate number of live cells.

5.2.1.1. If LCL are transduced with the Ad-LMP1-2 vector - send 2x10^6 transduced and non-transduced LCL for phenotyping if sufficient cell numbers.

5.2.2. Irradiate transduced LCL at 40Gy

5.2.3. Wash 3 times after irradiation

5.2.3.1. Centrifuge @ 400 g for 5 minutes

5.2.3.2. Resuspend in 10 mL complete media

5.2.4. After 3 washes resuspend LCL in complete media and perform live cell count using hemacytometer according to SOP E03.11

5.2.5. Centrifuge @ 400 g for 5 minutes, aspirate supernatant and resuspend at $2.5x10^5$ LCL per ml of CTL medium.

5.3. Cryopreservation of remaining CTLs

5.3.1. Centrifuge cells to be frozen at 400 x g for 5 minutes.

5.3.2. Transfer 5 ml of supernatant from centrifuged cells to a 15 ml centrifuge tube and store at -80°C for future QC testing. This supernatant can be discarded when all release criteria have been met for this CTL population.

5.3.3. Place cell pellet on ice for 10 minutes to cool.

5.3.4. Resuspend cells in freezing medium at no greater than 10^7 /ml.

5.3.5. Distribute aliquots of cells/freezing medium mixture into labeled cryotubes.

5.3.6. Place tubes in Nalgene freezing container and transfer to -80°C freezer and freeze overnight.

5.3.7. Transfer to the designated liquid nitrogen freezer for storage.

5.4. Thawing Transduced LCLs for CTL stimulation

5.4.1. Warm media to 37^o C

5.4.2. Thaw vial(s) of transduced LCL in 37^o C waterbath

5.4.3. Add thawed cells to warmed media

5.4.4. Centrifuge at 400 x g for 5 minutes.

5.4.5. Resuspend LCL in complete media or CTL media

5.4.6. Irradiate at 40Gy

5.4.7. Wash LCL twice

5.4.8. Count cells according to SOP E03.11

5.4.9. Centrifuge at 400 X G, aspirate supernatant

5.4.10. Resuspend LCL at $2.5x10^5$ LCL per ml of CTL medium.

5.4.11. LCL are now ready to stimulate CTLs.

6. Notes

6.1. ALL flasks and centrifuge tubes must be labeled with information to include patient name, patient number and component number. UNLABELED MATERIAL WILL BE DISCARDED.

6.2 NEVER work with more than one patient cell line at any one time.

6.3 ALWAYS use medium prepared and labeled specifically for each patient's cells. NEVER use medium to feed more than one patient's cells.

6.4 Perform all steps in a certified biological safety cabinet, using aseptic technique and following universal precautions.

7. Expected Results

7.1 Greater than 70% viability of transduced cells for use as antigen-presenting cells

8. References

8.1 Rooney CM, Smith CA, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation, *The Lancet* 1995; 345: 9-13.

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9. Review and Revisions

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