In vitro EBV Infection of Mononuclear Cells that Have Been Cryo-preserved

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[Abstract] Epstein-Barr Virus (EBV) is a B-lymphotropic herpesvirus which the majority of adult human population is latently-infected with. Various immunological and molecular *in vitro* studies have been facilitated by the use of EBV's ability to infect and transform B cells to immortalized polyclonal B cell lines. Many of these studies use freshly isolated cord-blood mononuclear cells (CBMC). Some experiments may, however, require EBV infection of samples that have been prospectively collected and cryo-preserved. Here we share a protocol that we used to successfully infect B cells from cryo-preserved CBMCs and peripheral-blood mononuclear cells (PBMC) (Sohlberg *et al.*, 2013; Saghafian-Hedengren *et al.*, 2013).

Materials and Reagents

1. Cryo-preserved CBMC
2. Cryo-preserved PBMC
3. EBV strain B95-8 containing supernatant
   Note: The batch here had a titer of 2.5 x 10^5 Ramos infectious units. RaIU, which was determined by infection of the EBV negative Burkitts lymphoma B-cell line Ramos, followed by anti-complement immunoflourescent assay (ACIF) to detect the number of infected cells.
4. RPMI 1640 (Life Technologies)
5. 10% heat-inactivated fetal-calf serum (Hyclone)
6. L-glutamine (2 mmol/l)

7. penicillin G-sodium (100 U/ml)
8. streptomycin sulfate (100 mg/ml) (Merck KGaA)
9. Complete cell-culture medium (see Recipes)

Equipment

1. 48-well flat-bottomed tissue-culture treated plates (Sarstedt AG)
2. Humified incubator with for adjustment of 37 °C and 5% CO_2 for cell culture

Procedure

Note about sample handling: Gentle and fast processing of samples during isolation of mononuclear cells contributes to higher B cell viability, which is central for successful in vitro infection.

1. Thaw CBMCs or PBMCs quickly at room temperature and wash two times with incomplete RPMI-1640 at 350 - 400 *x g* and 5-10 min at room temperature.
2. Determine cell numbers, and if applicable, divide cells to fractions that will be EBV infected and those that will serve as non-infected controls.
   Note: Remember that the cell numbers and concentrations in each well should be matched for the infected and non-infected fractions.
3. Wash an additional time with RPMI 1640.
4. For non-infected control cultures: Re-suspend cells in complete cell-culture medium to a concentration of 10^6 cells/ml and allocate to the appropriate wells in the plate. Incubate at 37 °C and 5% CO_2.
5. For EBV infection, discard supernatant completely and resuspend 10^6 cells per 100 µl B95-8 virus–containing supernatant in a small tube. Incubate at 37 °C and 5% CO_2 for 90 min with gentle mixing by swirling the tube every 30 min.
6. Wash the cells once with complete cell-culture medium, discard supernatant and re-suspended in complete cell-culture medium to a concentration of 10^6 cells/ml and then allocate to the appropriate wells in the plate.
7. Feed cells with complete medium on a weekly basis. The proportion of EBV-transformed B cells will increase with time and eventually these cells take over the entire culture. As we assessed the dynamics of NK and T cell and their functional capacity (Sohlberg *et al.*, 2013; Saghafian-Hedengren *et al.*, 2013) in our previous experimental settings, we found a 1-2 week-period of time suitable for this purpose.
8. The presence of EBV-infected cells can be confirmed by immunofluorescence staining for latent membrane protein-1 and EBV nuclear Ag 2 (Rasul *et al.*, 2012).

Representative data

Refer to Figure 1 in Sohlberg *et al.* (2013) for representative data on B cell characteristics following *in vitro* EBV infection of B cells.

Notes

1. Careful and fast processing of samples during isolation of mononuclear cells contributes to higher B cell viability, which is central for successful downstream *in vitro* EBV infection.
2. This protocol uses cells from EBV seronegative donors. Deplete T cells (by for instance magnetic-based methods to remove $CD3^+$ cells from StemCell™ or Miltenyi Biotech) prior to EBV *in vitro* infection in case samples are from EBV seropositive donors.

Recipes

1. Complete cell-culture medium
   10% heat-inactivated fetal-calf serum
   L-glutamine (2 mmol/l)
   Penicillin G-sodium (100 U/ml)
   Streptomycin sulfate (100 mg/ml)

Acknowledgments

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