

B LYMPHOCYTE IMMORTALIZATION

Description

Epstein-Barr virus (EBV) belongs to the herpesvirus family and it is the main agent that cause human mononucleosis. 95% approximately of adults are carriers of this virus and have high positive titer persistence through time. Lymphoblastoid marmoset cell line B95-8 (Callithrix genus) was established by the infection of marmoset B cells with EBV isolated from a patient with infectious mononucleosis. B95-8 cell line provides a source of EBV to generate continuous B lymphoblastoid lines from human donors. By DNA profiling, it has been confirmed that B95-8 cell line was derived from a cotton-top tamarin (*Saguinas oedipus*) instead of marmoset. This virus selectively infect B lymphocytes from a mixture of T, B and NK cells from peripheral blood lymphocytes (PBL) through complement receptor 2 (also known as CD21).

How does it work?

For B cell immortalization with EBV (Fig. 1) cells are isolated (day 0) from healthy donor or patient peripheral blood by density gradient. Isolated peripheral blood lymphocytes are resuspended in culture supernatant from B95-8 cell line (that contains the Epstein-Barr virus) in a 1:1 proportion with RPMI-1640 supplemented with 20% FBS, 1% glutamine, 1% antibiotic-antimycotic, and 20 µg/mL PHA. During the first two weeks after the infection, the culture is maintained once-twice per week with RPMI-1640, supplemented with 20% FBS, 1% glutamine and 1% antibiotic-antimycotic. Once lymphoblastoid clones are formed, cells need to be immunophenotyped to verify if they are CD19 positive, and after this confirmation, they can be subculture with complete medium with 10% FBS.

Advantages

This technique preserves functional genomes from B lineage and it is useful for:

- **B cell specific pharmacological preclinical assays.**
- **Preserve intrinsic B cell defects.**
- **Repository of genomic DNA and specific RNA to lineage B.**

Where has it been developed?

This technique has been developed in the Department of Immunology of the Faculty of Medicine of the Complutense University. The research group (<https://www.ucm.es/iao/t-cell-physiopathology>), in addition to collaborating in the immortalization of B lymphocytes, has consolidated a line of research on the pathophysiology of the human T lymphocyte, making significant contributions in the generation and characterization of in vitro models of the development and pathology of the T lymphocyte using HTLV-1, an immortalizing agent equivalent to the Epstein-Barr virus in B lymphocytes.

The B cells generated can help to:

- Have genetic material for detection of mutations when starting from scarce blood samples from patients with immunodeficiencies.
- Study the cellular and molecular basis of Common Variable Immunodeficiency and other clinical entities.

And moreover

This research group may offer the following additional services:

- Generation of T lymphocyte lines.
- Feasibility problems are solved.
- In vitro functional evaluation of the generated material. Pharmacological assays comparing with other lineages (B lymphocytes, epithelial, etc.)
- Cryopreservation and storage service (annual charge).
- Phenotyping of EBVs by flow cytometry (CD19, CD21, BCR...).
- Verification of *Mycoplasma* spp.-free cultures in the generated lineages.

Researcher in charge



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