Blymphocyte immortalization

Brief 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% antibioticantimycotic. 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.

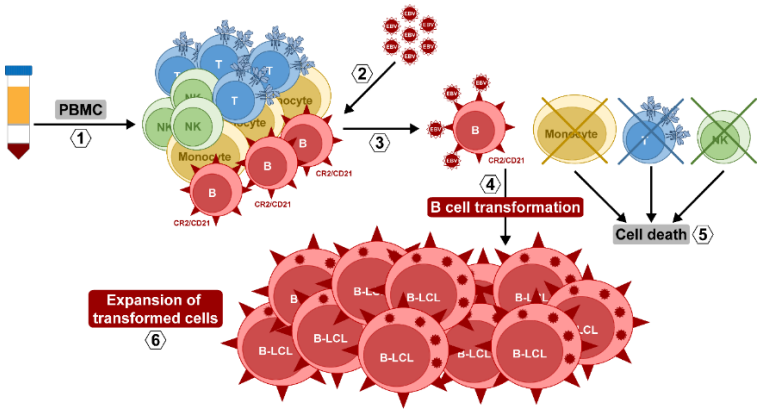

Figure 1: *B cell immortalization with Epstein-Barr virus. (1) Isolation of peripheral blood mononuclear cells (PBMC) by density gradient. (2) Addition of B95-8 culture supernatant containing the EBV. (3) EBV infect B cells specifically through CR2/CD21 receptor. (4) Once inside, EBV transform B lymphocytes into B-lymphoblastoid cell lines (B-LCL), while the rest of PBMC that have not been infected die. (5). (6) When they are transformed/immortalized, B-LCL proliferate and expand.*

What problem does it solve?

The **generated B cells** can contribute to:

- **Providing genetic material for mutation detection when working with limited blood samples from patients with immunodeficiencies.**
- **Serving as a cellular and molecular basis for Common Variable Immunodeficiency (CVID) research.**
- **Acting as models for studying diseases and the immune response, aiding in a better understanding of immunological mechanisms.**
- **Facilitating the study of immune memory, allowing researchers to analyze B lymphocyte responses to pathogens or vaccines.**

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What future products will it develop?

The **immortalization of B lymphocytes** has multiple applications in biomedicine, biotechnology, and research:

- Continuous production of monoclonal antibodies, enabling the generation of specific antibodies for research, diagnostics, and treatments in diseases such as cancer, autoimmune disorders, and infections.
- Storage of B lymphocytes from patients with rare diseases, allowing for long-term studies and personalized research.
- Immunotherapies for treating cancer and other diseases, contributing to development of therapeutic approaches.

Competitive advantages compared to other research

The **immortalization of B lymphocytes** has several competitive advantages over other methodologies in biomedical research and immunology, including:

- Unlimited Antibody Production: Immortalized B lymphocytes can grow indefinitely, allowing for continuous and stable antibody production. This contrasts with techniques such as extracting sera from animals or patients, which are limited and prone to variability.
- Cell Biobanks: This technique enables the long-term storage of patient cells, making personalized treatments possible. In contrast, direct B lymphocyte extraction methods do not allow for long-term preservation.
- Greater Reproducibility in Studies: Since these cells are clones, experiments can be conducted with consistent results. This is an advantage over studies using primary cells or sera, which may introduce variability in outcomes.
- Lower Costs and Higher Efficiency in Antibody Production: Compared to immunizing animals for antibody extraction—a longer and more expensive process—this method is more cost-effective and efficient.

Where has it been developed?

This technique has been developed in School of Medicine Immunology Dpt of Complutense University. The research group, apart from collaborate immortalizing B cells, has consolidated a research topic based in human T cell physiopathology, with significant publications in the generation and characterization of in vitro models of the development and pathology of T lymphocytes using Herpesvirus saimiri (HVS), an immortalizing agent similar to that in Epstein-Barr virus with B cells. **Immortalized B cells can help to:**

- *Have genetic material for the detection of mutations from inmunodeficient patients when blood samples are scarce.*
- *Detect the cellular and molecular base of Common variable Immunodeficiency (CVID). (Cabanillas et al. Inmunología 1998; 17: 93- 100)*

And moreover…

This research group offers the following additional services:

- **Immortalized Th y Tc cell line genration**
- **Viability problems solutions**
- **In vitro functional evaluation of the material generated. Pharmacological assays comparing other lineages (B cells, peithelial cells, …)**
- **Cryopreservation service**

Researcher in charge

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