B CELL IMMORTALIZATION

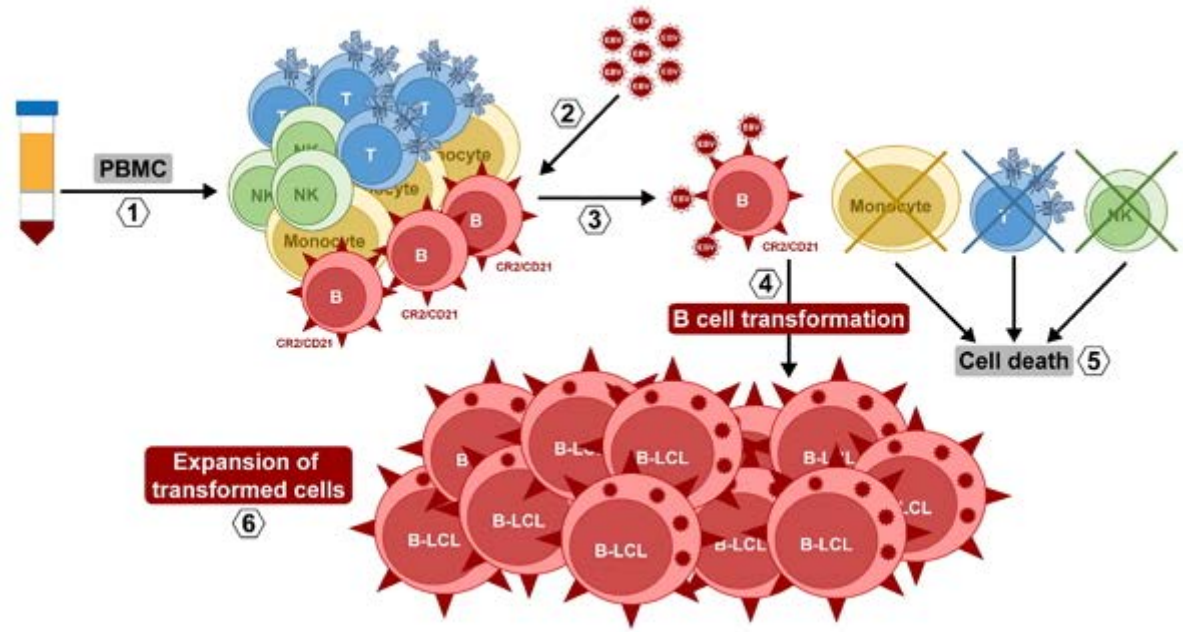
Description

B cell immortalization

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 actually 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 with a density gradient using Ficoll (GE Healthcare). 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 (Lonza) supplemented with 20% FBS, 1% glutamine, 1% antibiotic-antimycotic (Gibco), and 20 µg/mL PHA (Sigma-Aldrich). 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.



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.*

Advantages

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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 Immunology Department, at the Faculty of Medicine of Complutense University of Madrid. 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 *HTLV-1*, 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).

And also

This research group offers the following additional services:

- **Generation of T lymphocyte lines**
- **Viability problems solutions**
- **In vitro functional evaluation of the material generated. Pharmacological assays comparing other lineages (B cells, peithelial cells, …)**
- **Cryopreservation service**

Responsible Researcher

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