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Isolation of Primary Mice Splenocytes For *In Vitro* Research

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ABSTRACT

Animal cell culture is of great importance in research. We isolated mouse splenocytes from Swiss albino mice by using a mechanical method and cultured them in medium with fetal bovine serum, 1% penicillin-streptomycin and 1% amphotericin as antibiotic and an antifungal, but without mitogen. The cells were confluent after 72 h, and their viability, measured by trypan blue assay, was 95%. The splenocytes were passaged four times and no fungal or bacterial contamination was observed during carrying out the experiment.

1. INTRODUCTION

The spleen is the largest lymphoid organ of the body and has hematological and immunological functions. It is a unique lymphoid organ that plays a critical role in homeostasis of the immune and hematopoietic systems (Wang *et al*., 2019). It stores and purifies erythrocytes, metabolizes hemoglobin, and recycles iron. It also provides a important function for the immune system by mounting a primary immune response to antigens in the blood and synthesizing antibodies (Mebius and kraal, 2005). Splenocytes are one of the white blood cell types situated in the spleen or purified from splenic tissue. Splenocytes consist of a variety of cell populations such as mononuclear cells derived from the spleen, T-lymphocytes, B-lymphocytes, NK-cells, and NK T-cells. Primary mouse splenocytes can be used to isolate CD4+ T-cells, CD8+ T-cells and CD45R+ B cells (Berrington *et al*., 2005). Splenocytes are used for a variety of assays, including T-cell activation, proliferation in response to mitogens, and cytokine production. Moreover, mouse splenocytes can be used for a wide variety of immunology-based applications. *In-vitro* immunoassays, such as mixed lymphocyte reactions, cytotoxicity assays to measure antibody-dependent cellular cytotoxicity, and plaque assays are routinely performed using these cells (Grizzle and Polt, 1988). In addition, mouse splenocytes are also used in *ex vivo* applications, such as characterization of cell populations and generation of genetically modified cells through retroviral infection. Moreover, several studies were carried out on mouse splenocytes as models for testing plant extracts, apoptotic and toxicological studies. This study aimed to establish solenocytes from mouse spleen for *in vitro* culture to be used for different experimental purposes.

2. MATERIAL AND METHODS

The study was carried out with the permission of the local ethics committee. Specific-pathogen-free Swiss albino mice, typically 6-8 weeks old, were bred under specific pathogen free conditions at the Animal Health Department, Tripoli, Libya. All methods were carried out in accordance with the approved guidelines and regulations.

2.1 Isolation of splenocytes

Spleen were removed aseptically, placed in Petri dishes with 5 ml of RPMI 1640 medium containing 10% of FBS fetal bovine serum and 1% penicillin-streptomycin and gently quashed between glass slides.

Cells and tissue fragments were dispersed by repeated pipetting then transferred to a chilled centrifuge tube and kept at 4°C. The procedure was repeated three to four times on the larger pieces of tissue in the dish, with fresh PBS, until most of the fragments were dispersed. Larger fragments were allowed to settle in the tube; the suspension of single cells and smaller aggregates was pipetted through several layers of sterile gauze (10.16 by 10.16cm) into a second chilled centrifuge tube and sedimented at 4000 rpm for 10 min at 4 C. The supernatant fluid was decanted, and the pellet was suspended in1 ml of PBS. A cell count was performed with white blood cell diluting fluid. Erythrocytes were lysed in ammonium chloride hemolysis buffer (0.8% NH_4Cl and 0.1 mM EDTA) and then washed twice in complete RPMI-1640 medium and then once in PBS containing 2% FBS and 5 mM EDTA.

2.2. Medium and culture conditions

The maintenance and sub culturing of the splenocytes was carried out in a Class II biosafety cabinet laminar flow (Euroclone Bioair, Italy). The splenocytes were cultured at a density of $1.5x10^6$ cells/ml in complete medium as described, 5 ml spleen culture were cultured in six 25-cm loosely capped bottles. Splenocytes were grown in RPMI-1640 medium (Gibco) with 15% fetal bovine serum (Sigma Aldrich) and 1% penicillin-streptomycin (Gibco) and incubated at 37°C in a humidified atmosphere of 5% CO_2 and relative humidity of 95%.

2.3 Cell death assay

Cell survival was measured using the trypan blue dye exclusion technique by mixing 100 µl of cell suspension with an equal volume of a 0.4% trypan blue solution and counting clear (live) and blue (dead) cells.

2.4 Detection of microbial contamination

The cultures were periodically examined under the microscope for fungal or bacterial contamination. Moreover, the cell supernatant was cultured in Maconky and blood agar at 37°C in terms of bacteria contamination suspected and potato dextrose agar media in terms of fungus contamination for 14 days at 22°C. Splenocytes were examined daily with an inverted microscope (Prism Optical) and images obtained by a digital camera.

3. RESULTS AND DISCUSSION

The spleen is the largest lymphoid organ, with important functions in innate and adaptive immune responses (Wu *et al,* 2004, Mebius *et al,* 2005). Mouse spleen cells are the most commonly used cell population for immune studies in mice because the spleen is easily removed and provides a relatively large number of cells. We isolated splenocytes as a primary cell culture and tested them *in vitro*. Figure 1 a showed the splenocytes offered from Science Cell research Laboratories Company has been used to demonstrate the similarity shape of splenocytes among commercial available brand producer and splenocytes prepared locally at BTRC lab. Figure 2 illustrated the splenocytes appeared round and with intact cell membranes and they were free of erythrocytes. They showed no abnormality and were comparable to commercially available solenocytes (Figure 1). Moreover, 95% of them were viable. Previous study reported that, one of the earliest and most common methods for measuring cell viability is the trypan blue staining assay (Taghavi and Yazid, 2015). The use of viability days for estimation of cell viability has been commonly relied upon in experimental procedure using cell culture *in vitro* (Hutz *et al.,* 1985). In culture, the cells became about 80% confluent after 72 h. In figure 2, Splenocytes were appeared healthy and well divided with 10 % FBS. Moreover, figure 3 showed the splenocyte during mitotic division particularly in telophase as we can see cells were attached by new cell membrane and appeared in pairs pre separated gradually to new individual single cells, and yields cell suspensions with a high viability rate. Furthermore, microbial contamination is one of the major challenges of cell culture experiments since the source of culture contamination are hard to identify and eliminate (Nardon, 2007). In current work we did not observe any bacterial or fungal contamination through four passages, as indicated by the absence of turbidity or other physical changes in the splenocytes culture medium, in contrast to the positive test control. These results demonstrated that the newly-established mice primary solenocytes cells were not contaminated by bacteria or fungi, Sub culturing cell suspension of splenocytes was carried out in our lab until the fourth passage.

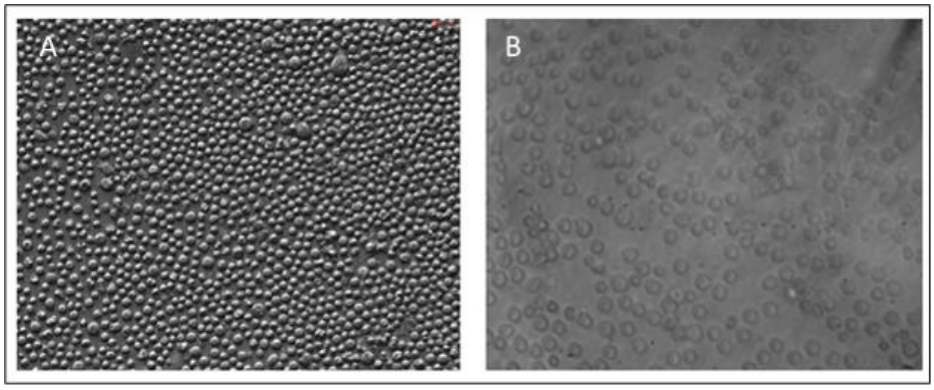

Figure 1. (A) Commercial available splenocytes (B) splenocytes isolated from mice spleen locally (magnification x100).

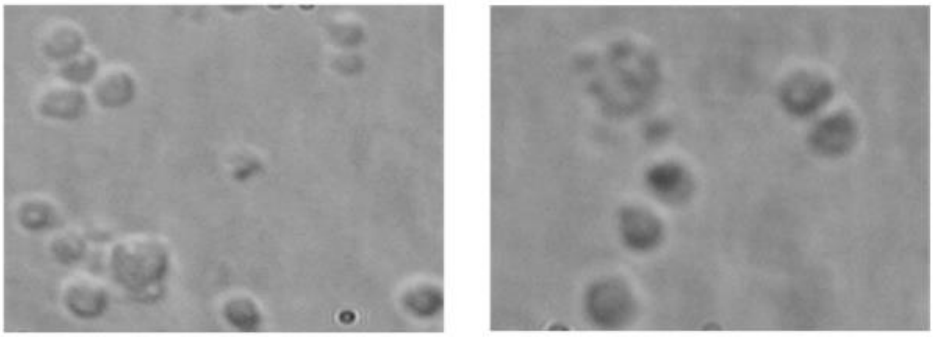

Figure 2. Phase-contrast micrograph (magnification x100) illustrating the development of splenocytes after 24 h of culture.

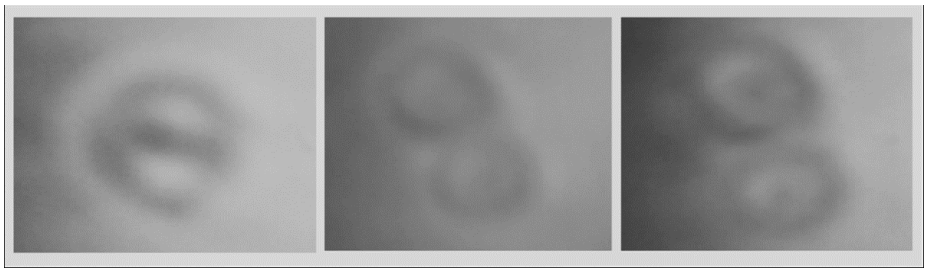

Figure 3. Lymphocytes during telophase isolated from mice splenocytes (magnification x 400)

4. CONCLUSION

In summary, in this study splenocytes were isolated from mice spleen by mechanical method succeefully. To our knowledge, for the first time, we cultured a primary cell culture from mice spleen which would provide a suitable tool for Cytological, Immunological, Pathological and pharmacological investigations.

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