Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

A Simple Method to Immortalize Human Leukocytes and its Potential Applications

Elida Cleyse Gomes da Mata and Kanzaki L.I.B*

Laboratory of Bioprospection, Department of Pharmacy, University of Brasília. Brasília, DF. Brazil.

***Correspondence:**
Kanzaki L.I.B, Laboratory of Bioprospection, Department of Pharmacy, University of Brasília. Brasília, DF, Brazil.

Received: 25 March 2019; **Accepted:** 24 April 2019

Citation: Elida Cleyse Gomes da Mata, Kanzaki L.I.B. A Simple Method to Immortalize Human Leukocytes and its Potential Applications. Microbiol Infect Dis. 2019; 3(2): 1-3.

ABSTRACT

The immunological response to any antigen is identified and quantified "in vivo" but the tools to perform "in vitro" the same procedures in cell culture are cumbersome and inaccurate. Usually lymphocytes are isolated by centrifugation in sucrose density gradient and cultured in RPMI medium requiring stimulation with phytohemagglutinin and maintained by the addition of interleukin-2 in the medium. Besides, "in vitro" assays to evaluate any natural or synthetic compound could be masked by the mitogen and IL-2 activity. An easy and practical approach to culture human leukocytes and evaluate the response to any natural compound or the immunological response to any pathogen is described. Instead of just lymphocyte isolation, all leukocytes were collected after separation from total peripheral blood cells in sucrose density gradient. Leukocytes in culture, representing different cell phenotypes, mimic "in vitro" cell cooperation during host exposition to foreign antigens. Therefore, the procedure described utilizes the filtered supernatant of lymphoblastic cell lines, HUT-78, to activate primary human leukocytes to divide and differentiate, maintained indefinitely under culture. In this way "in vitro" stimulated leukocytes with any immunogenic compound respond producing the corresponding cytokines, chemokines and antibodies. Among other applications, viral production could be achieved including viral isolation. A large array of potential diagnostic and therapeutic applications could be devised utilizing this simple and new method.

Keywords

Leukocytes, Cell transformation, Cytokines, Virus production, Biotechnology.

Introduction

Cell culture methodology has been utilized for a century and innumerous applications in diagnostic procedures of pathogens detection as also immunological and therapeutic protocols are broadly applied in the spectra of biotechnology [1-3].

Animal utilization in experimental biology raises ethical issues and animal rights activists protests all around the world demanding the implementation of brand new technologies in order to ban animals from any experiment. Certainly cruelty with the animals advocates in favor of "*in vitro*" models to all fields of experiments in biomedicine [4,5].

Computer science allied to statistics add great effort to life science in order to understand vital mechanisms to prevent, treat and cure human and animal diseases as also applicable in the field of botany [6,7].

Methodological approaches to isolate retroviruses and to assess antiretroviral activity of natural products require the use of "*in vitro*" systems, mainly employing lymphoblastic cell lines expressing retrovirus receptors [8-11]. Usually the established cell lines utilized are monoclonal which support retrovirus replication and could be suitable models for antiretroviral screening of natural products [12,13]. Therefore, the direct action of natural products compound in the retrovirus infectious cycle could be accessible but, other mechanisms of action are based on the induction of antiviral restriction factors produced by the infected cell, and cell signalling is a major event that takes place to ultimately evoke the response. A new and simple method is described here which collect all human primary leukocytes and maintain them indefinitely in culture.

Methods

Leukocytes isolation

Heparinized total peripheral human blood in 3 mL volume is gently layered over 3 mL of sucrose density gradient (Histopaque 1070, Sigma) in a 15 mL conical centrifuge tube at room temperature. After 30 minutes at 400 x g centrifugation, all clear supernatant is recovered and washed 3 times in RPMI incomplete medium (Sigma) at 250 x g for 10 minutes. Sedimented cells were ressuspended in RPMI medium supplemented with 10 % fetal bovine serum (Gibco) and 1% penicillin/streptomycin solution (Sigma).

Immortalization process

The Human cutaneous T cell lymphoma derived from peripheral blood of a patient with Sezary syndrome [12] (Gazdar et al., 1980) denominated HUT-78 cell line, was kindly donated by the NIH AIDS reagents (https://www.aidsreagent.org/reagentdetail.cfm?t=cell_lines&id=461). Cells were cultivated in complete RPMI 1640 medium and after cell metabolism, indicated by the yellow acidic color developed, cells were centrifuged at 250 x g for 5 minutes and supernatant collected and filtered in 0.22 um pore device. The primary human leukocytes were fed with 50% RPMI complete medium and 50 % filtered supernatant of HUT-78 cells also cultured in RPMI medium supplemented with fetal bovine serum and antibiotics.

Cell microscopy

Cells were cultivated in small plates (12.5cm^2, 25mL) and observed under light inverted microscope.

Discussion

Initially cultivated human primary leukocytes presented 12-15 µm diameter. The majority of the cells remained suspended in culture medium and showed round morphology. Few cells attached to the plate surface and displayed a macrophage star-like appearance with discrete cytoplasm extensions. After 72 hs time elapsed since HUT-78 cell supernatant addition, the primary leukocytes changed their morphology to bigger cells, about triple the size viewed in the initial culture. These cells also lost the roundness appearance, appearing more pleomorphic.

Preliminary data (unpublished data) demonstrated the response of transformed human leukocytes under stimulation with HUT-78 cell supernatant to produce cytokines after treatment with natural products likewise plant extracts or animal antigens. Previously those same natural products were utilized to treat HUT-78 cell lines which poorly responded by measuring cytokines production. Certainly the cooperation among different cell populations composing the leukocyte pool as T and B lymphocytes, granulocytes (neutrophils, eosinophils, basophils and mast cells) and monocytes, that process the natural product as antigen and through the monocyte differentiation into macrophages and the coexistence of circulating dendritic cells, assure the presentation of antigens to T and B lymphocytes, besides different subsets of these cells exerting distinct role according to the immune system aims [14-16]. All network established by the heterogeneous leukocyte population allow the cells to cooperate and promptly respond to any treatment by antigens [17].

Virions production by the transformed leukocytes is easily achieved if virus receptors are expressed in any of the different cell population of leukocytes, e.g. human lentivirus and deltaretrovirus [18,19]. Established lymphoblastic cell lines are more restricted concerning the expression of virus receptors while leukocyte cultures have a broad spectra of virus receptors [20,21].

The addition of HUT-78 supernatant to the leukocyte culture did not interfere in any of the experiment above mentioned as cell controls without treatment were utilized for comparison. It was not yet determined which factor the HUT-78 cell supernatant produces to immortalize leukocytes, but it could be assumed that the same growth factors that induced leukemia or lymphoma [21] in patients from whom the lymphoblastic cell cultures were established, are present in the HUT-78 supernatant added to primary human leukocytes that induced them to be immortalized. Many cycles of freezing and defreezing in 10 % DMSO and 90 % fetal bovine serum solution were carried out and the immortalized leukocyte cells always proliferated, supporting many passages without changing the established morphology. In addition, the leukocyte supernatant after filtration in 0.22 µm exhibited the same action on fresh new human peripheral leukocytes as originally done with HUT-78 cell culture.

Conclusion

Many applications are devised utilizing this simple and fast technology as mentioned and also, to evaluate human patients suffering from different diseases by checking up the patient's leukocyte response to any antigen analyzing the cytokine and chemokine profile. Monoclonal antibodies could be either produced utilizing the transformed leukocytes by previously priming the cells before the immortalization process. Another potential application would be the transformed leukocyte mass culture to produce a desired cytokine/chemokine and after well characterization of factors present in HUT-78 supernatant, it would be possibly the utilization of produced cytokine/chemokine in the patient from whom the leukocytes were isolated.

Coming and ongoing experiments aiming to identify the growth factors that constitute HUT-78 cell supernatant and well characterize the transformed leukocytes under HUT-78 cell supernatant action, carrying out cell karyotyping, cell supernatant screening for antibody activity and cell phenotyping of different populations would drive this new and simple methodology of leukocyte methodology to a large array of therapeutic, diagnostic and biotechnological purposes.

Acknowledgments

This research work was conducted with funds from the Foundation for Scientific Research Support in the Federal District, Brazil (FAP/DF, Brazil). We are also indebted to NIH AIDS Reagents Program, US for kind donation of HUT-78 cell line. The methodology described here was submitted to patent by the Center

of Technological Development, University of Brasilia (CDT/UnB), Brasilia, DF.

References

1. Yao T, Asayama Y. Animal-cell culture media: History, characteristics, and current issues. Reprod Med Biol. 2017; 16: 99-117.
2. Jazayeri SH, Amiri-Yekta A, Bahrami S, et al. Vector and Cell Line Engineering Technologies Toward Recombinant Protein Expression in Mammalian Cell Lines. Appl Biochem Biotechnol. 2018; 185: 986-1003.
3. Phelps J, Sanati-Nezhad A, Ungrin M, et al. Bioprocessing of Mesenchymal Stem Cells and Their Derivatives: Toward Cell-Free Therapeutics. Stem Cells Int. 2018; 23.
4. Fernandes MR, Pedroso AR. Animal experimentation: A look into ethics, welfare and alternative methods. Rev Assoc Med Bras. 2017; 63: 923-928.
5. Cheluvappa R, Scowen P, Eri R. Ethics of animal research in human disease remediation, its institutional teaching; and alternatives to animal experimentation. Pharmacol Res Perspect. 2017; 5.
6. Wu Y, Wang G. Machine Learning Based Toxicity Prediction: From Chemical Structural Description to Transcriptome Analysis. Int J Mol Sci. 2018; 19: pii: E2358.
7. Dalio RJD, Herlihy J, Oliveira TS, et al. Effector Biology in Focus: A Primer for Computational Prediction and Functional Characterization. Mol Plant Microbe Interact. 2018; 31: 22-33.
8. Bekut M, Brkić S, Kladar N, et al. Potential of selected Lamiaceae plants in anti (retro) viral therapy. Pharmacol Res. 2018; 133: 301-314.
9. McCarron A, Donnelley M, McIntyre C, et al. Challenges of up-scaling lentivirus production and processing. J Biotechnol. 2016; 240: 23-30.
10. Gutiérrez-Granados S, Farràs Q, Hein K, et al. Production of HIV virus-like particles by transient transfection of CAP-T cells at bioreactor scale avoiding medium replacement. J Biotechnol. 2017; 263: 11-20.
11. da Mata ÉC, Mourão CB, Rangel M, et al. Antiviral activity of animal venom peptides and related compounds. J Venom Anim Toxins Incl Trop Dis. 2017; 23: 3.
12. Gazdar AF, Carney DN, Bunn PA, et al. Mitogen requirements for the *in vitro* propagation of cutaneous T-cell lymphomas. Blood. 1980; 55: 409-417.
13. Roos JW, Maughan MF, Liao Z, et al. LuSIV cells: a reporter cell line for the detection and quantitation of a single cycle of HIV and SIV replication. Virology. 2000; 273: 307-315.
14. Costa S, Bevilacqua D, Cassatella MA, et al. Recent advances on the crosstalk between neutrophils and B or T lymphocytes. Immunology. 2019; 156: 23-32.
15. Wałajtys-Rode E, Dzik JM. Monocyte/Macrophage: NK Cell Cooperation-Old Tools for New Functions. Results Probl Cell Differ. 2017; 62: 73-145.
16. Jamin C, Achour A, Youinou P, et al. Regulatory lymphocytes: a new cooperation between T and B cells for a better control of the immune response. Presse Med. 2014; 43: 18-26.
17. Schuijs MJ, Hammad H, Lambrecht BN. Professional and 'Amateur' Antigen- Presenting Cells In Type 2 Immunity. Trends Immunol. 2019; 40: 22-34.
18. Woodham AW, Skeate JG, Sanna AM, et al. Human Immunodeficiency Virus Immune Cell Receptors, Coreceptors, and Cofactors: Implications for Prevention and Treatment. AIDS Patient Care STDS. 2016; 30: 291-306.
19. Shimauchi T, Piguet V. DC-T cell virological synapses and the skin: novel perspectives in dermatology. Exp Dermatol. 2015; 24: 1-4.
20. Fukudome K, Furuse M, Fukuhara N, et al. Strong induction of ICAM-1 in human T cells transformed by human T-cell-leukemia virus type 1 and depression of ICAM-1 or LFA-1 in adult T-cell-leukemia-derived cell lines. Int J Cancer. 1992; 52: 418-427.
21. Bunn PA Jr, Foss FM. T-cell lymphoma cell lines (HUT102 and HUT78) established at the National Cancer Institute: history and importance to understanding the biology, clinical features, and therapy of cutaneous T-cell lymphomas (CTCL) and adult T-cell leukemia-lymphomas (ATLL). J Cell Biochem Suppl. 1996; 24: 12-23.