



ORIGINAL ARTICLE

Expression Pattern of Interferon- γ in Human Leukemic T Cell Lines Following Treatment with Phytohemagglutinin, phorbol myristate acetate and Lipopolysaccharide

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ABSTRACT

Background: As a T helper type 1 (Th1) derived cytokine, Interferon gamma (IFN- γ) is an important regulator of inflammatory immune responses. Furthermore, IFN- γ plays an essential role in defense against tumors and intracellular pathogens. This study was designed to assess the pattern of IFN- γ production in human leukemic (Jurkat and Molt-4) T cell lines in vitro.

Methods: Jurkat and Molt-4 cells were cultured in whole RPMI-1640 media. The cells were imbedded at a density of 2×10^6 cell/ml. The cells were stimulated with different concentrations of Phytohemagglutinin (PHA) (2-10 μ g/ml), phorbol myristate acetate (PMA) (1-25 ng/ml) or lipopolysaccharide (LPS) (1-4 μ g/ml) for activation and cytokine production for 48 hours. Then the cell-conditioned media were used for IFN- γ assay. Analysis of variance (ANOVA) was done for comparing the groups statistically.

Results: PHA and PMA substantially augmented IFN- γ level in human leukemic T cells (Molt-4 and Jurkat) in a dose-dependent manner after 48 hours of incubation compared with untreated control cells, whereas LPS did not have any significant effect on IFN- γ production in human leukemic T cell lines compared with unstimulated cells.

Conclusion: human leukemic Jurkat and Molt-4 T cell lines could potentially produce IFN- γ with different amounts. PHA was a more potent stimulator of IFN- γ production than PMA. Molt-4 cell line could produce more IFN- γ than Jurkat cell line. These cells could be appropriate for studying the mechanisms of action of immunomodulators as well as screening the IFN- γ stimulators/inhibitors.

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Introduction

Interferons (IFNs) are well-studied proinflammatory cytokines.^{1,2} Interferon gamma (IFN- γ), as a T helper type 1 (Th1) derived cytokine is an important regulator of inflammatory immune responses.³ Furthermore, IFN- γ plays an essential role in defense against tumors and intracellular pathogens.^{4,5} There are also crucial roles suggested for IFN- γ in the pathogenesis of numerous diseases such as vitiligo, alopecia areata and Type 1 diabetes.^{6,7} Dysregulation of IFN- γ in some diseases

including intracranial aneurysms and kawasaki disease have been demonstrated.^{8,9} Besides decrease of IFN- γ in several diseases such as complicated respiratory viral infections, HIV, cystic fibrosis and asthma has been shown.¹⁰⁻¹³ In addition increased level of IFN- γ in various disorders including type 2 diabetes and inflammatory bowel disease (IBD) has been reported.^{14,15} Immunomodulatory effects of physical activity on IFN- γ has also been revealed.^{16,17} Moreover, the beneficial effects of some drugs such as propranolol, pentoxifylline and

methotrexate in a number of cardiovascular diseases such as autoimmune myocarditis has been partly attributed to their inhibitory effects on IFN- γ production.^{18,19} Upregulation of IFN- γ by some immunomodulators such as piperine, clinacanthus nutans (a medicinal plant) and leptin had therapeutic effects on immune-compromised tuberculosis, hepatoma and leishmania donovani infections, respectively.²⁰⁻²²

In this study, pattern of IFN- γ production in human leukemic (Jurkat and Molt-4) T-cell lines has been evaluated in vitro.

Materials and Methods

Microtiter plates, tubes and flasks were purchased from Nunc (Falcon, USA). Fetal calf serum (FCS) got from Gibco (USA). Phytohemagglutinin (PHA), Phorbol myristate acetate (PMA), Lipopolysaccharide (LPS), penicillin, streptomycin, RPMI-1640 and trypan blue (TB) were purchased from sigma (USA). IFN- γ standard ELISA kit was obtained from R&D company (USA). Human leukemic Molt-4 (NCBI C149) and Jurkat (NCBI C121) T cells were purchased from the National Cell Bank of Iran (NCBI). These cells were retained in RPMI-1640 media complemented by 10% FCS in 5% CO₂ at 37°C.

Cell Culture

The leukemic cells were cultivated in RPMI-1640 media added with 10% FCS, penicillin (100 IU/ml) and streptomycin (100 μ g/ml) at 37°C in 5% CO₂. After that the cells were distributed at a concentration of 2×10^6 cell/ml and next treated with different concentrations of PHA (1-10 μ g/ml), PMA (5-25 ng/ml) or LPS for 48 hours. The supernatants of cell culture media were removed and used for IFN- γ quantification. Each test was arranged in triplicates.²³

IFN- γ Test

The extent of IFN- γ produced in cell culture supernatants of leukemic T cells (Molt-4 and Jurkat) was measured with the Quantikine human enzyme-linked immunosorbent

assay (ELISA) kits (R&D organizations) as per the manufacturer's procedures. This test uses the quantifiable sandwich enzyme immunoassay method. Whole RPMI media was used also for control. In order to illustrate the standard curves, human recombinant IFN- γ was used.

Statistical Analysis

IFN- γ measurement in cell-culture medium was done in three diverse tests and the data were specified as mean \pm standard error of the mean (SEM). Analysis of variance (ANOVA) was used for statistical analysis between groups. $P < 0.05$ was statistically designated as significant. For statistically significant variations, test of multiple comparison of Tukey was performed (5%).

RESULTS

Pattern of PHA-Induced IFN- γ Production in Molt-4 Cells

IFN- γ production was relatively low in unstimulated human leukemic Molt-4 cells, but PHA considerably increased IFN- γ production in Molt-4 cells following 48-hour treatment compared with the control cells dose-dependently ($P < 0.05$, figure 1).

Pattern of PHA-Induced IFN- γ Production in Jurkat Cells

IFN- γ production was very low in unstimulated human leukemic Jurkat cells but PHA markedly increased IFN- γ production in leukemic Jurkat cells subsequent to 48-hour treatment compared with the control cells. The PHA-stimulated IFN- γ production in leukemic Jurkat cells was concentration-dependent ($P < 0.05$, figure 1).

Pattern of PMA-Induced IFN- γ Production in Molt-4 Cells

Unstimulated human leukemic Molt-4 cells produced relatively little amount of IFN- γ , but again PMA considerably increased IFN- γ production in Molt-4 leukemic cells following 48-hour treatment compared with the control cells dose-dependently ($P < 0.05$, figure 2).

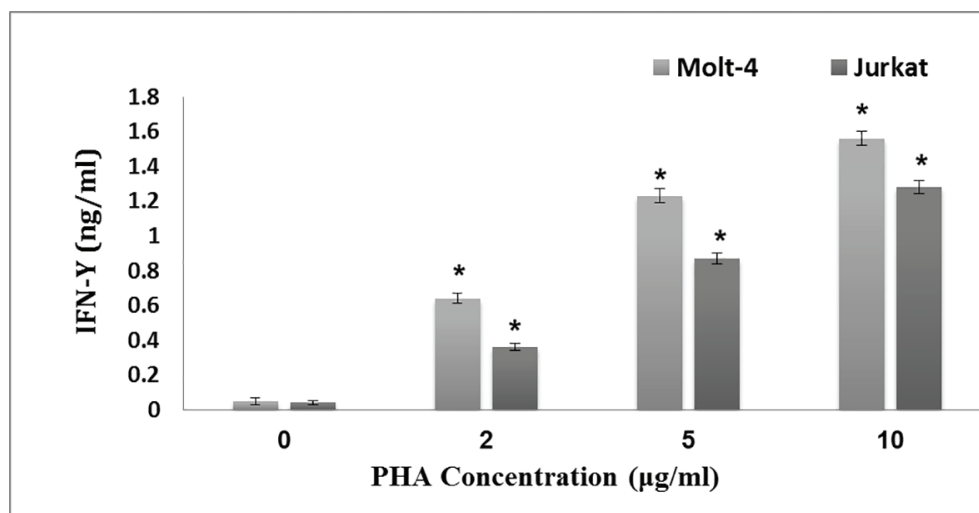


Figure 1: Effect of PHA on IFN- γ secretion by human leukemic Jurkat and Molt-4 T cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of phytohemagglutinin (PHA) (2-10 μ g/ml) for 48 hours. Finally, IFN- γ levels in cell culture media was quantified by ELISA kit. Results are mean \pm SEM of three distinctive tests. * $P < 0.05$ was specified significant.

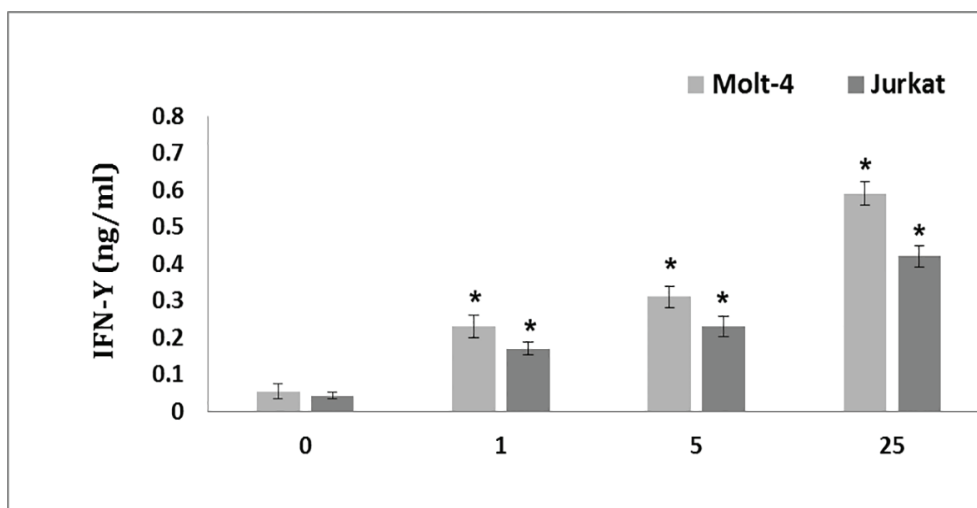


Figure 2: Impact of PMA upon IFN- γ secretion by human leukemic Molt-4 and Jurkat T-cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of phorbol myristate acetate (PMA) (1-25 μ g/ml) for 48 hours. Finally, IFN- γ levels in cell culture media was quantified by ELISA kit. Results are mean \pm SEM of three distinctive tests. * $P < 0.05$ was specified significant.

Pattern of PMA-Induced IFN- γ Production in Jurkat Cells

Unstimulated human leukemic Jurkat cells produced very low amounts of IFN- γ but PMA considerably enhanced IFN- γ production in leukemic Jurkat cells following 48-hour treatment compared with the control cells. The PMA-stimulated IFN- γ production in leukemic Jurkat cells was concentration-dependent ($P < 0.05$, figure 2).

Pattern of LPS-Induced IFN- γ Production in Molt-4 Cells

IFN- γ production was relatively low in unstimulated human leukemic Molt-4 cell line and LPS had no effect on IFN- γ production following the 48-hour treatment compared with unstimulated control cells (figure 3).

Pattern of LPS -Induced IFN- γ Production in Jurkat cells

IFN- γ production was very low in unstimulated human leukemic Jurkat cells and LPS had no effect on IFN- γ production in these cells subsequent to the 48-hour treatment compared with unstimulated control cells (figure 3).

Discussion

In this study, we found that Jurkat and Molt-4 leukemic T cell lines can produce IFN- γ . We also found that PHA/PMA increase IFN- γ production in above mentioned leukemic cells but LPS did not show any significant effect on IFN- γ production. Different patterns of IFN- γ are expressed in different diseases²⁴⁻²⁶ and different profiles of IFN- γ are produced in response to various

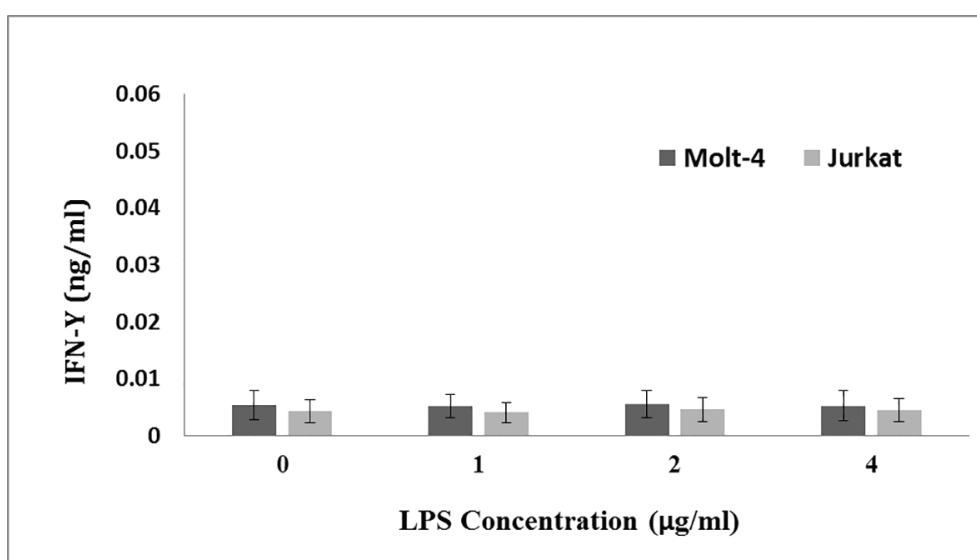


Figure 3: Impact of LPS on IFN- γ secretion by human leukemic Molt-4 and Jurkat T-cell lines. These cells (2×10^6 cells/ml) were cultured in complete RPMI-1640 medium and next were stimulated with various doses of lipopolysaccharide (LPS) (1-4 μ g/ml) throughout 48 hours. Finally, IFN- γ levels in cell culture media was quantified by ELISA kit. Results are mean \pm SEM of three distinctive tests. * $P < 0.05$ was specified significant.

antigens.²⁷⁻²⁹ In the present study, PMA, PHA and LPS were used to assess their modulatory effects on IFN- γ production. Consistent to our results, induction of IFN- γ mRNA expression by PHA and PMA in Jurkat cells have been shown by Benbernou et al.³⁰ In their study, Jurkat cells were used at 1×10^5 cells per well and were stimulated simultaneously by PHA (10 μ g/ml) and PMA (10 ng/ml). However, Benbernou and colleagues assessed IFN- γ mRNA expression and did not evaluate the level of IFN- γ production. We used 2×10^6 Jurkat or Molt-4 cell and stimulated the cells with different concentrations of PHA (1-10 μ g/ml) or PMA (1-25 ng/ml).

According to our results, LPS did not display any significant effect on IFN- γ production in human leukemic cells (Jurkat and Molt-4). Different effects of LPS on IFN- γ production has been determined by different studies.^{31,32} In Kabanov and colleagues' study, *Rhodobacter capsulatus* PG LPS stopped IFN- γ production in human whole blood.³¹ Their findings are consistent with our results. We assessed the LPS effect on the unstimulated human leukemic cells (Jurkat and Molt-4) and since these cells produced very low amounts of IFN- γ in unstimulated state, therefore we could not conclude that LPS does not have any effect on IFN- γ production. It is suggested to assess the effects of LPS on IFN- γ production on cells stimulated by PHA or PMA. In another study, LPS-stimulated bone marrow mesenchymal stromal cells, showed profoundly decreased IFN- γ gene expression while co-cultured with T lymphocyte.³²

In our study PMA increased IFN- γ production in leukemic cells dose-dependently after 48 hours of stimulation. This enhancing effect reached the maximum point at 25 ng/ml of PMA. In accordance to our results, another study showed an increase of IFN- γ production in rat whole blood cells dose-dependently which maximized 6 hours after stimulation with 25 ng/ml PMA.³³ The reason for the discrepancy between our study and the mentioned study was that we used leukemic T-cells incubated with PMA for 48 hours while the mentioned whole blood cells incubated with PMA for 0-10 hours. In addition, consistent with our data, Barten et al. have shown an augmented IFN- γ secretion in healthy whole blood cells stimulated with 25 ng/ml PMA after 5 hours.³⁴ Moreover, similar to our study, Keski-Nisula and colleagues revealed that PMA increased the excretion of IFN- γ in healthy whole blood cells after 24 hours incubation.³⁵

Furthermore, contrary to a previous study³³ which did not observe any significant influence of PHA on the production of IFN- γ in rat whole blood cells, in our study PHA profoundly increased IFN- γ production in human leukemic T cells in a dose-dependent manner after 48 hours of incubation. This discrepancy between that study and ours may be due to the difference in cells which were used, species and incubation time. We used human leukemic T-cells incubated with PHA for 48 hours while in the mentioned study, the researchers used rat whole blood cells incubated with PHA for 0-10 hours.

Taken together, according to the results of our study, diverse profiles of IFN- γ are produced in response to various stimulating agents. Also we found that PHA

and PMA are potent stimulators of IFN- γ production in human leukemic T-cells. Moreover, PHA was a more potent stimulator of IFN- γ production than PMA. In addition, Molt-4 cell line could produce more IFN- γ than Jurkat cell line. Hence these cells may be appropriate tools to evaluate the mechanism of IFN- γ induction in diseases in which IFN- γ production is dysregulated as well as screening the regulators, stimulators or inhibitors of IFN- γ induction.

Conclusion

According to our data, human leukemic Jurkat and Molt-4 T cell lines could potentially produce IFN- γ with different amounts. PHA was a more potent stimulator of IFN- γ production than PMA. In addition, Molt-4 cell line could produce more IFN- γ than Jurkat cell line. Therefore, these cells possibly will offer proper tools to assess the regulating mechanisms of IFN- γ secretion in diseases in which IFN- γ production is dysregulated as well as screening of the regulators, stimulators, or inhibitors of IFN- γ production.

Conflict of Interest: None declared.

References

1. Mathew DJ, Lucy MC, D Geisert R. Interleukins, interferons, and establishment of pregnancy in pigs. *Reproduction*. 2016; 151(6):R111-22. doi: 10.1530/REP-16-0047. PubMed PMID: 27001998.
2. Stifter SA, Bhattacharyya N, Pillay R, Flórido M, Triccas JA, Britton WJ, et al. Functional Interplay between Type I and II Interferons Is Essential to Limit Influenza A Virus-Induced Tissue Inflammation. *PLoS Pathog*. 2016; 12(1):e1005378. doi: 10.1371/journal.ppat.1005378.
3. DiNardo AR, Mandalakas AM, Maphalala G, Mtetwa G, Mndzebele T, Ustero P, et al. HIV Progression Perturbs the Balance of the Cell-Mediated and Anti-Inflammatory Adaptive and Innate Mycobacterial Immune Response. *Mediators Inflamm*. 2016; 2016:1478340. doi: 10.1155/2016/1478340. PubMed PMID: 27006526.
4. McNamara MJ, Hilgart-Martiszus I, Barragan Echenique DM, Linch SN, Kasiewicz MJ, Redmond WL. Interferon- γ production by peripheral lymphocytes predicts survival of tumor-bearing mice receiving dual PD-1/CTLA-4 blockade. *Cancer Immunol Res*. 2016; 4(8):650-7. doi: 10.1158/2326-6066.CIR-16-0022. PubMed PMID: 27262113.
5. Blauenfeldt T, Wagner D, Aabye M, Heyckendorf J, Lange B, Lange C, et al. Thermo stability of IFN- γ and IP-10 release assays for latent infection with *Mycobacterium tuberculosis*: A TBnet study. *Tuberculosis (Edinb)*. 2016; 98:7-12. doi: 10.1016/j.tube.2015.04.013. PubMed PMID: 27156612.
6. Rork JF, Rashighi M, Harris JE. Understanding autoimmunity of vitiligo and alopecia areata. *Curr Opin Pediatr*. 2016; 28(4):463-9. doi: 10.1097/MOP.0000000000000375. PubMed PMID: 27191524.
7. Vaseghi H, Jadali Z. Th1/Th2 cytokines in Type

- 1 diabetes: Relation to duration of disease and gender. *Indian J Endocrinol Metab.* 2016; 20(3):312-6. doi: 10.4103/2230-8210.180002. PubMed PMID: 27186546.
8. Zhang HF, Zhao MG, Liang GB, Yu CY, He W, Li ZQ, et al. Dysregulation of CD4(+) T Cell Subsets in Intracranial Aneurysm. *DNA Cell Biol.* 2016; 35(2):96-103. doi: 10.1089/dna.2015.3105. PubMed PMID: 26667180.
9. Lee SB, Kim YH, Hyun MC, Kim YH, Kim HS, Lee YH. T-Helper Cytokine Profiles in Patients with Kawasaki Disease. *Korean Circ J.* 2015; 45(6):516-21. doi: 10.4070/kcj.2015.45.6.516. PubMed Central PMCID: PMC4661368.
10. Ivanova ON, Argunova EF, Alekseev SN, Ystugina TV, Varfolomeev AR, Troev IP, et al. Adaptive mechanisms of the immune system in children in far north. *Wiad Lek.* 2015; 68(4):534-6. PubMed PMID: 26887130.
11. Twizerimana AP, Mwatha J, Musabyimana JP, Kayigi E, Harelimana Jde D, Karanja SM, et al. Immunological profiles in HIV positive patients following HAART initiation in KIGALI, RWANDA. *East Afr Med J.* 2014; 91(8):261-6. PubMed PMID: 26862650.
12. Zhou MM, Chu XP, Yang HZ, Kou F, Zhao AH, Jia W. [Anti-inflammatory and anti-allergic effects of acidic fraction of Pheratima extract in asthma mice induced by ovalbumin]. *Zhongguo Zhong Yao Za Zhi.* 2008; 33(19):2249-52. PubMed PMID: 19166019.
13. Nunes FB, Castro MC, Silva TM, Araújo RN, Becker HM, Crosara PF, et al. Cytokine profile in subjects with Cystic Fibrosis and nasal polyposis compared to patients with no nasal disorders. *Braz J Otorhinolaryngol.* 2010; 76(1):25-8. PubMed PMID: 20339685.
14. Mahmoud F, Al-Ozairi E. Inflammatory cytokines and the risk of cardiovascular complications in type 2 diabetes. *Dis Markers.* 2013; 35(4):235-41. doi: 10.1155/2013/931915. PubMed Central PMCID: PMC3782813.
15. He Y, Lin LJ, Zheng CQ, Jin Y, Lin Y. Cytokine expression and the role of Th17 cells in mice colitis. *Hepatogastroenterology.* 2012; 59(118):1809-13. PubMed PMID: 23115792.
16. Palmefors H, DuttaRoy S, Rundqvist B, Börjesson M. The effect of physical activity or exercise on key biomarkers in atherosclerosis--a systematic review. *Atherosclerosis.* 2014; 235(1):150-61. doi: 10.1016/j.atherosclerosis.2014.04.026. PubMed PMID: 24835434.
17. Tuon T, Souza PS, Santos MF, Pereira FT, Pedrosa GS, Luciano TF, et al. Physical Training Regulates Mitochondrial Parameters and Neuroinflammatory Mechanisms in an Experimental Model of Parkinson's Disease. *Oxid Med Cell Longev.* 2015; 2015:261809. doi: 10.1155/2015/261809. PubMed PMID: 26448816.
18. Hajighasemi F, Mirshafiey A. In Vitro Effects of Propranolol on T Helper Type 1 Cytokine Profile in Human Leukemic T Cells. *Int J Hematol Oncol Stem Cell Res.* 2016; 10(2):99-105. PubMed Central PMCID: PMC4888155.
19. Han L, Guo S, Wang Y, Yang L, Liu S. Experimental drugs for treatment of autoimmune myocarditis. *Chin Med J (Engl).* 2014; 127(15):2850-9. PubMed PMID: 25146626.
20. Sharma S, Kalia NP, Suden P, Chauhan PS, Kumar M, Ram AB, et al. Protective efficacy of piperine against *Mycobacterium tuberculosis*. *Tuberculosis (Edinb).* 2014; 94(4):389-96. doi: 10.1016/j.tube.2014.04.007. PubMed PMID: 24880706.
21. Huang D, Guo W, Gao J, Chen J, Olatunji JO. *Clinacanthus nutans* (Burm. f.) Lindau Ethanol Extract Inhibits Hepatoma in Mice through Upregulation of the Immune Response. *Molecules.* 2015; 20(9):17405-28. doi: 10.3390/molecules200917405.
22. Dayakar A, Chandrasekaran S, Veronica J, Maurya R. Leptin induces the phagocytosis and protective immune response in *Leishmania donovani* infected THP-1 cell line and human PBMCs. *Exp Parasitol.* 2016; 160:54-9. doi: 10.1016/j.exppara.2015.12.002. PubMed PMID: 26688099.
23. Hajighasemi F, Hajighasemi S. Effect of propranolol on angiogenic Factors in Human Hematopoietic Cell Lines in vitro. *Iran Biomed J.* 2009; 13(4):223-8. PubMed PMID: 19946348.
24. Rodríguez-Cortés A, Carrillo E, Martorell S, Todolí F, Ojeda A, Martínez-Flórez A, et al. Compartmentalized Immune Response in Leishmaniasis: Changing Patterns throughout the Disease. *PLoS One.* 2016; 11(5):e0155224. doi: 10.1371/journal.pone.0155224. PubMed PMID: 27171409.
25. Wang F, Cai R, He D, Zhao Y, Ye Y, Zhang X. Serum IFN- γ -inducible chemokines CXCL9 and CXCL10 are elevated in non-immediate drug hypersensitivity reactions. *Asian Pac J Allergy Immunol.* 2016; 34(3):236-41. doi: 10.12932/AP0679. PubMed PMID: 27001652.
26. König K, Klemens C, Haack M, Nicoló MS, Becker S, Kramer MF, et al. Cytokine patterns in nasal secretion of non-atopic patients distinguish between chronic rhinosinusitis with or without nasal polys. *Allergy Asthma Clin Immunol.* 2016; 12:19. doi: 10.1186/s13223-016-0123-3.
27. Huang Q, Yu W, Hu T. Potent Antigen-Adjuvant Delivery System by Conjugation of *Mycobacterium tuberculosis* Ag85B-HspX Fusion Protein with Arabinogalactan-Poly (I:C) Conjugate. *Bioconjug Chem.* 2016; 27(4):1165-74. doi: 10.1021/acs.bioconjchem.6b00116.
28. Joshi SS, Arankalle VA. Differential Immune Responses in Mice Immunized with Recombinant Neutralizing Epitope Protein of Hepatitis E Virus Formulated with Liposome and Alum Adjuvants. *Viral Immunol.* 2016; 29(6):350-60. doi: 10.1089/vim.2016.0024. PubMed PMID: 27285290.
29. Haga A, Takahashi E, Inomata Y, Kawahara K, Tanihara H. Differentiated Expression Patterns and Phagocytic Activities of Type 1 and 2 Microglia. *Invest Ophthalmol Vis Sci.* 2016; 57(6):2814-23. doi:

- 10.1167/iov.15-18509. PubMed PMID: 27227350.
30. Benbernou N, Esnault S, Shin HC, Fekkar H, Guenounou M. Differential regulation of IFN- γ , IL-10 and inducible nitric oxide synthase in human T cells by cyclic AMP-dependent signal transduction pathway. *Immunology*. 1997; 91(3):361-8. PubMed Central PMCID: PMC1364004.
 31. Kabanov DS, Serov DA, Zubova SV, Grachev SV, Prokhorenko IR. Dynamics of Antagonistic Potency of *Rhodobacter capsulatus* PG Lipopolysaccharide against Endotoxin-Induced Effects. *Biochemistry (Mosc)*. 2016; 81(3):275-83. doi: 10.1134/S000629791603010X. PubMed PMID: 27262197.
 32. Sangiorgi B, De Freitas HT, Schiavinato JL, Leão V, Haddad R, Orellana MD, et al. DSP30 enhances the immunosuppressive properties of mesenchymal stromal cells and protects their suppressive potential from lipopolysaccharide effects: A potential role of adenosine. *Cytotherapy*. 2016;18(7):846-59. doi: 10.1016/j.jcyt.2016.04.004. PubMed PMID: 27260206.
 33. Ai W, Li H, Song N, Li L, Chen H. Optimal method to stimulate cytokine production and its use in immunotoxicity assessment. *Int J Environ Res Public Health*. 2013; 10(9):3834-42. doi: 10.3390/ijerph10093834. PubMed Central PMCID: PMC3799516.
 34. Barten MJ, Rahmel A, Bocsi J, Boldt A, Garbade J, Dhein S, et al. Cytokines analysis to predict immunosuppression. *Cytometry A*. 2006; 69(3):155-7. doi: 10.1002/cyto.a.20215. PubMed PMID: 16479614.
 35. Keski-Nisula L, Roponen M, Hirvonen MR, Heinonen S, Pekkanen J. Stimulated cytokines production correlates in umbilical arterial and venous blood at delivery. *Eur Cytokine Netw*. 2004; 15(4):347-52. PubMed PMID: 15627644.