http://pmjournal.ir Original Article

Autumn 2023, Volume8, Issue 31 (51 -54)

Proinflammation and Inflammatory Cytokine Gene Expression Changes in Human Macrophages Infected by L. majorl

Khodaberdi Kalavi[1,2*], Ghorbanmohammad Kochaki[1], Kamran Eghbalpour[3], Nahid Eghbalpour[3]

[1]Department of Clinical Laboratory Science, Laboratory Science Reseach Center, Golestan Research Center of Gastroenetrology & hepatology, Golestan, Iran.
[2]Department of clinical Laboratory Sciences, Laboratory Science Research Center, Golestan Research Center of Gastroenterology and Hepatology
[3]Department of Biology, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

DOI: 10.22034/pmj.2023.2018677.1027

**Corresponding author: Khodaberdi Kalavi, Department of Clinical Laboratory Science, Laboratory Science Reseach Center, Golestan Research Center of Gastroenetrology & hepatology. Email: kalavi@goums.ac.ir.*

Submitted: 2023-12-23
Accepted: 2023-12-30

Keywords:
Proinflammation
Macrophages
L.major
Immune response
RNA-Seq

©2023.Personalized Medicine Journal

Abstract:
Background: Leishmania Species produced diseases include clinical problems from cutaneous self-limiting to severe non-healing forms such as visceral leishmaniasis (VL). As an obligatory intracellular parasite these pathogens proliferate and survive inside macrophages in animals and human; while these cells as a major host immune cell destroy majority of disease producing agents. Because macrophages act as first line of innate immunity, produce several molecules when activated. Proinflammatory and inflammatory cytokines are produced by these cells through their activation, act as main coordinators of the immune system against pathogens and other harmful disease producing factors against the body. Through such a mechanism the immune response resolves the problem. To play such a critical role many cells as monocytes, macrophages, DCs and others involved in T cell regulation to establish proper innate and adaptive immunity responses. Proinflammatory and inflammatory cytokines are produced in a network acting through many signal pathways.
Methods: In this descriptive designed study, quality-controlled cDNA samples sequenced (RNA-seq) and mapped against a standard human genome version.
Results: Based on the results of this study, proinflammatory and inflammatory gene expressions were significantly upregulated.
Conclusion: Upregulations of proinflammatory and inflammatory gene expressions early infection time might be an indication for an early innate immunity response.

INTRODUCTION

Leishmaniasis is a worldwide health problem caused by *Leishmania species* as an intracellular obligate protozoan that proliferate and survive inside a harsh environment. Leishmaniasis clinically manifests as cutaneous, mucocutaneus and visceral forms. Cutaneous forms are the most prevalent, and distributed almost in all parts of the world. In Iran it's prevalent in north east parts as Golestan province, parts of Khorasan, Esfahan and Kerman provinces that the principle infective parasite types are *L. major and L. tropica species*. Recently some cases of visceral cases have reported from parts of Iran.

Leishmania parasites are very smart and make subversions and alterations on protective potentials of the host immune cells to be able to proliferate and survive inside them. For this, much times researchers faced with the questions such as "Why the host immune cells get infected and let the parasite to easily proliferate and survive inside them and produce the disease?" and to answer this type questions many studies have been carried out and because macrophages as one of the most potent involved host immune cells, majority of studies have focused on immune response against the parasite and clarifying of metabolic changes following infection.

Following inoculation of the parasite by the insect (sand fly) it enters into the blood and are attached by complement system proteins and get destroyed up to 90 present (1) and then pursued to be phagocytized by neutrophils and macrophages. Neutrophils have few life spans and few hours later destroyed after infection and released promastigotes together with neutrophil residues are phagocytized by macrophages and dendritic cells (DCs) (2). Phagocytosis of parasite elements by neutrophils known as "Trojan horse" (3)

Copyright © The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org /lic enses/by /4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

and some in vivo studies on mice demonstrate that this is a way to production of more severe clinical problem; while in absence of such a phenomenon disease production capacity of the pathogen is not more severe (4). It seems that through such a process the parasite better learns how to make a hostile environment to proliferate and survive.

In order to better controlling of the pathogen, innate immunity primarily reacts and then adaptive immunity involved. Immune cells as neutrophils, macrophages, DCs, natural killer cells (NK cells) and critical molecules as cytokines and complement system are some elements of innate immunity. In fact neutrophils and macrophages are in first line when *leishmania species* infect animals and human. LipoPhosphoGlycan (LPG) and a zinc dependent protease (gp63) are two surface proteins present in *leishmania species* and have shown to be very effective tools of disease production by the pathogen (5, 6). Some receptors on macrophages recognize them to response to produce soluble molecules as cytokines and triggering of other molecules as complement factors.

Parasitic infective tools are as proteolytic saliva factors acting as facilitating tool of skin penetration and gp63 and LPG proteins that act to provide the inside of host immune cells a favored environment to replicate and survive. A well-known of such mechanisms cleavage potency of gp63 of *leishmania spp*.

The LACK (*Leishmania* homologue of receptors for activated C kinase) as a known studied parasitic antigen triggers the host immune response (7). This response including the controlling of the pathogen from skin contact through its blood stream trip, phagocyte membrane contacts with an interest to be phagocytized, phagolysosome formation, conversion of infective form of promastigotes into proliferating amastigote forms. Immune response includes immediate innate immunity followed by adaptive immunity. In this context cells of innate immunity as neutrophils, macrophages, DCs and Natural Killer Cells (Nk cells) with of their cytokines and for the adaptive immunity CD4 T-helper cells (Th-1, Th-2 cells) and related cytokines and receptors are well studied.

This study presents was presented Proinflammatory and inflammatory cytokine gene expression alterations as results of early innate immunity response to human macrophages infected by L.major four hour post infection time (4hpi) in vitro.

MATERIAL AND METHODS

This was a descriptive study of RNA-seq data on human monocyte-derived macrophages infected by L. major to determine gene expression alterations on infected macrophages . Peripheral blood mononuclear cells (PBMC) isolated by MACS (Magnetic Activated Cell Sorting) method, Milteny Germany). Macrophages prepared from pure monocytes' culture in RPMI1640 medium (Roswell Park Memorial Institute) + 10% of FBS (Fetal bovine serum) + 1% of Pen/Strep (Penicillin/Streptomycin) and 20ng M-CSF (Monocyte Colony Stimulation Factor-1) in 8-10 days. Iranian strain of Leishmania major (MRHO/IR/75/ER) were incubated in RPMI 1640 supplemented with 10% FBS, 1% pen/str and gentamycin for 3-4 days at 22-25 C. Promastigotes in stationary phase of parasite life cycle, isolated and co-cultured with 5-7 promastigotes per macrophage. Additional non-infected macrophages and macrophages co-cultured with silicone micro beads (Latex particles sized 4.1µ) in presence of monocyte –colony stimulating factor (M-CSF) for 4 hours considered as controls.

RNA extraction was done by use of Trizol reagent (Invitrogen) and stored at -75c˚ [negative75c˚] based on the manufacturer protocol. Complementary DNA (cDNA) synthesis performed (select sure, Agilent USA, 2017). Quality controlled cDNA samples sequenced using MiniSeq Illumina machine (End Single Reads) and then resultant Fast Q files trimmed (Trimmomatic Bolger, 2014). Determination of uniquely and multi mapped reads was done against human and *L.major* genomes (hg38*[GRCh38]* and Lmjf.01.760). DE (differential expression) analysis performed using Dseq2 analytical method. For gene annotation we used bioDBnet-Biological Database network. For log2 fold change conversion we used base 2logarythm log2 calculator (WWW.endmeno.com/algebra/log2. Php.

RESULTS

Transcriptome changes expressed in human macrophages infected by *L.major spp*., were profiled using RNA-seq. CD14 (Cluster of Differentiation 14) monocytes that were obtained from healthy volunteer donors (Golestan province transfusion center), converted to macrophages and infected by *L.major* metacyclic promastigotes 5-7 parasites per macrophage. Non polarized macrophages (intact) were incubated in parallel with parasite and micro beads (4.1µ) and macrophage alone. After incubation time of 4hrs, total RNAs from 3x3 incubated macrophages series isolated and following assessment of quality controlling of the samples and storage, RNA-seq was done.

Results of RNA-seq showed up regulation of pro and inflammatory cytokine genes indicative of an early innate immunity response. Significantly upregulated Proinflammatory and related gene expressions were *IL-1α and IL-1β, IL-6, IL8, TNFα, IL-27 and IL-15; however, IL-18, IL-23, IL-12 and IFNγ* gene expressions were high in infected macrophages with

of no/ or minimal expressions in control samples.

DISCUSSION

Proinflammatory and inflammatory cytokine expressions is an early immune response reaction and may be an indication for macrophage activation for controlling and monitoring of the evaded invaded pathogens. According to the studies cytokine genes as *IL-1a, IL-1b, TNF-a, IFNγ* are up regulated involving in macrophage activation and NO production via iNOS expression inside phagolysosome (8). Most of these cytokine genes expression upregulation was significant [$p<0.05$]. MAPK (Mitogen Activated Protein Kinase) gene expression was insignificantly downregulated. These mitogen activation that based on several reports expressed by some *L.Spp*, induces to prevent factors responsible to NO production [Nitric Oxide] from iNOS [inducible Nitric oxide synthase] by infected macrophages (9). Expression patterns of TNF/a, IFNγ are reported to be in high and moderate levels in mice and human. Higher levels extracted from kinds of skin ulcers demonstrates deeper and more non healing lesions than moderate expression patterns (10).

IL-1α, β and TNFα cytokine genes were up regulated significantly. *IFNγ gene* expression that is mainly secreted by Th-1 cells, NK cells to effect on ROS (Radical Oxygen Species) production to enhance respiratory burst to killing intracellular pathogens. In vitro studies demonstrated that it may be also produced under effects of IL-12 and IL-18 by monocyte derived macrophages treated with M-CSF as done in this study and also activated alveolar macrophages secrete IFNγ following treatment using combination of IL-12 and IL-18 (11). Based on in vivo studies the effect of IFNγ in leishmaniasis is enhancement of respiratory burst inhibition in non-activated macrophages (12).

IL-1 (α, β) cytokine genes both subtypes were significantly up-regulated. This factor is a pro-inflammatory expressing cytokine gene in early infections and inflammatory diseases. It's believed that like TNFα both act as protective roles against infections derived from kinds of pathogens. In intracellular based diseases as leishmaniasis, it acts as cross road between protection and worsening of the skin lesions in L.major infection by development of Th-17 related immunosuppressive responses (13).

IL-6 and IL-8(CXCL-8) cytokine gene expressions were highly upregulated in this study. IL-6 is produced by a wide spectrum of cell types appearing in acute and chronic inflammatory states. It has been previously known as hepatocyte stimulating factor for acute phase protein production from liver (14). Howler it's known as one of the most activated protein of the phenomenon known as "cytokine storm "that considered as to be suppressed in some clinical states. IL-6 acts as a pleiotropic cytokine in leishmaniasis and studies show its effcts on both Th-1 and Th-2 immune responses that could act as a factor of immune activation and suppression roles (15).

IL-8 is a neutrophil chemo-attractive factor and studies demonstrated that this chemokine is responsible to make several cells of innate immunity recruit to the site of infection early infection time (16). In course of leishmaniasis and infective roles of *L.Spp*, the smart parasite is rapidly phagocytized by neutrophils and some modulations and reprogramming processes are related to this process and for this it's called as "Trojan horse". Because of low life span of neutrophils they become destroyed and as the result body faces with a huge amount of dead neutrophil structural residues. Therefore it may provide more time for macrophages to eliminate and hence to make macrophages to become weaker and to provide an opportunity to well reprogramming of the pathogen to infect and survive (17).

IL12, 23 and 27 that share similar structural and functional homologies, showed upregulated gene expressions (18). These cytokines are produced by some cell kinds especially activated macrophages. The role of these are as resistance against majority of *L.Spp*, including Th-1` response and inhibition of suppressive Th-2 responses (19).

IL-15 cytokine gene expression was significantly upregulated; while its homolog IL-2 showed few replicates in test sample and did not show any replicates in two control samples.

IL4, 10, 13, 22 … as immunosuppressing/modulator cytokines and released from Th-2 immune cell subtypes and act mainly as infection susceptible factors, have not expressed in this study. The main reason for this probably is related to the absence of such cells that almost 100 present were eliminated due to macrophage preparation from monocyte isolation method [MACS].

CONCLUSION

According to the results of this study we can conclude that pro-inflammatory and inflammatory cytokine gene expressions from macrophages infection by *L.major* mostly are overexpressed early infection time points. It also showed that these factors are main markers of innate immune responses against L.major infection through phagocytosis, phagolysosome formation and principally intracellular events to innate and adaptive immunity cross road to monitor and control the invaded pathogen.

There is no conflict of interest.

REFERENCES

1.Ana Caroline Costa-da-Silva, Danielle de Oliveira Nascimento , Jesuino R. M. Ferreira, Kamila Guimarães-

Pinto, Leonardo Freire-de-Lima, Alexandre Morrot, et al. Immune Responses in Leishmaniasis: An Overview. Trop. Med. Infect. Dis. 2022.7(4): 1-16.
2.Dong Liu and Jude E. Uzonna. The early interaction of leishmania with macrophages and dendretic cells and its influence on the host immune response. Frontiers in cellular and infection microbiology.2012.2(5):1-8.
3.Mariana M. Chaves l, Sang Hun Lee, Olena Kamenyeva Kashinath Ghosh l, Nathan C. Peters. DaviSacks. The role of dermis resident macrophages and their interaction with neutrophils in early establishment of leishmania major infection transmitted by sand fly bite. Plus pathogens. 2020 November 2: 1-24.
4.Joao Carlos Araujo, Alba Valeria Machado da Silva. The Role of Neutrophils in the Interaction with Leishmania: Far beyond a Simple Trojan horse? Open journal of Animal Sciences. 2021. 11(7): 399-421.
5.Edoardo Torres-Guerrero , Marco Romano Quintanilla-Cedillo ,Julieta Ruiz-Esmenjaud , Roberto Arenas. Leishmaniasis: a review [version 1; referees: 2 approved]. F1000Research. 2017; 6(2): 1-15.
6.Aretha ChanID Jose-Mauricio Ayala, Fernando Alvarez, Ciriaco Piccirillo,George Dong, David Langlais, Martin OlivierID. The role of Leishmania GP63 in the modulation of innate inflammatory response
To Leishmania major infection. PLOS ONE. 2021; 16(12): 1-21.
7.David Sacks and Nancy Noben-Trauth. The Immunology of susceptibility and resistance to Leishmania major in mice. Nature Reviews, Immunology. 2002; 2(11): 845-858.
8.Vincenzo Bronte, Paola Zanovello. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol. 2005; 5(8): 641-654.
9.Dong Liuand Jude E.Uzonna. The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune response. Frontiers in Cellular and Infection Microbiology. 2012; 2(3): 1-8
10.Nahid Maspi , Amir Abdoli , Fathemeh Ghaffarifar. Pro- and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. Pathogens and Global Health. 2016; 110(6): 251-260.
11.Laila Darwich, Gemma Coma,Ruth Pen˜a, Rocio Bellido, Ester J.J. Blanco, Jose´ A. Este, Francesc E. Borras, Bonaventura Clotet,Lidia Ruiz, Antoni Rosell, Felipe Andreo, R. Michael E. Parkhouse and Margarita Bofill. Secretion of interferon-c by human macrophages demonstrated at the single-cell level after costimulation with interleukin (IL)-12 plus IL-18. Immunology. 2008; 126(3): 386–393.
12.Phillip Scott and Fernanda O. Novais. Cutaneous leishmaniasis:Immune responses in protection and pathogenesis. Nature reviews, Immunology. 2016; 16(6): 581-592.
13.Elena Voronov, Shahar Dotan, Lubov Gayvoronsky, Rosalyn White1, Idan Cohen. IL-1-induced inflammation promotes development of leishmaniasis in susceptible BALB/c mice. International Immunology. 2010; 22(4): 245–257.
14.Toshio Tanaka, Masashi Narazaki, and Tadamitsu Kishimoto. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol. 2014;6:a016295. 1-16.
15.Bhavnita Soni, Bhaskar Saha, Shailza Singh. Systems cues governing IL6 signaling in leishmaniasis. Cytokine. 2018; 105(3): 169-175.
16.R Badolato 1, D L Sacks, D Savoia, T Musso. Leishmania major infection of human monocytes induces expression of IL-8 and MCAF. Exp parasitol. 1996; 82(1): 21-26
17.Ger Van Zandbergen, Matthias Klinger, Antje Mueller, Sonja Dannenberg, Andreas Gebert, Werner Solbach, Tamás Laskay. Cutting edge: Neutrophil granulocyte serves as a vector for Leishmania entry into macrophages. Journal of immunology. 2004; 173(11): 6521-6525.
18.Dario A A Vignali and Vijay K Kuchroo. IL-12 family cytokines: immunological playmakers. Nauture Immunology. 2012; 13(4): 722-728.
19.Gee K, Guzzo C, Che Mat NF, Ma W, Kumar A. The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflamm Allergy Drug Targets. 2009 Mar;8(1):40-52.