Original Article

Anti-leishmaniasis Effect of *Staphylococcus Aureus* Protein A on the Size of the Lesion and Parasitic Load

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Abstract

Background: Many studies in the past have evaluated the role of immune system boosters in the treatment of leishmania major infection. Protein A (PA) is one of the structural components in peptidoglycan cell wall of gram-negative bacteria such as staphylococcus aurous which functions as a stimulator in the cellular immune system. The present study aims to evaluate the anti-inflammatory effect of PA on the recovery of leishmania major infection.

Materials and Methods: This study was conducted on 24 female Balb/c-infected mice. The experimental group received PA at a dose of 60 mg/kg for four weeks. There was no intervention for the negative control group; the third group received the solvent of PA and sterile H2O; and the positive control group received Amphotericin B at a dose of 1 mg/kg body weight. At the end of the treatment period, a real-time polymerase chain reaction (PCR) assay was performed to determine parasitic burden, and the size of the lesions was measured by caliper with an accuracy of 0.01 mm.

Results: Results showed that PA did slightly decrease the wound spread and growth but not to an extent that can be considered statistically significant. Also, differences in cycle threshold (Ct) values between the treated group and the untreated group was not impressive.

Conclusions: Although findings showed that PA isn't such a good candidate for leishmania treatment, it may still be suitable for therapies that use multiple drugs in combination to speed up the healing of leishmaniosis, an issue that merits evaluation in future studies.

Keywords: Protein A, leishmania major, staphylococcus aurous

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Submitted: 03-Jan-2021; **Revised:** 29-Aug-2021; **Accepted:** 02-Feb-2022; **Published:** 21-Mar-2023

Introduction

Leishmaniasis has been reported as a parasitic and tropical disease in as many as 98 countries.[1] This is one of the most common human and animal infections.[2] The most common form of the disease is cutaneous leishmaniasis, which leads to serious disabilities due to ulcers in different areas of the body.[3] The long life of the parasite is found in macrophages. The most effective anti-leishmania response in the macrophage is the production of reactive oxygen (ROS) and nitrogen (RNS) species.[4,5] Staphylococcal protein A (PA) as a virulence agent is always found in clinical purifications.[6]

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Quick Response Code:

Website: www.advbiores.net

DOI: 10.4103/abr.abr_291_20

The molecular weight of this protein is about 45-60 KD. During staphylococcal infection, the mature polypeptide sequence of this protein is cut after cleavage and placed at the cell wall through the X domain, located at the carboxyl terminus. This protein contains 4 to 5 immunoglobulin-binding domains (E-D-A-B-C), including 56 to 61 amino acids per domain. The domains are very similar to each other, and each domain consists of three alpha helixes.[7]

PA as a superantigen can produce a wide range of immune and biological responses.[8] It can stimulate innate immune responses as a pathogenic factor. Previous studies' findings

How to cite this article: Tavalaei Z, Zeinalian M, Khanahmad H, Hejazi H. Anti-leishmaniasis effect of *Staphylococcus aureus* protein A on the size of the lesion and parasitic load. Adv Biomed Res 2023;12:61.

showed that PA can be seen in pathogen-associated molecular patterns (PAMPs) that are recognized by specific receptors on the surface of phagocytes like macrophage and dendritic cells and consequently trigger innate immune responses. Two main mechanisms of innate immune responses induced by PAMPs are the development of inflammation and the antiviral function in the intracellular and extracellular microbes. These strategies can lead to the removal of damaged cells and help repair the tissue initiated by signals and molecules released from damaged cells. The inflammation is caused by the infiltration of different leucocytes, secreted cytokines and chemokines, and soluble plasma proteins at the site of infection. The presence of molecules emerging from the activation of innate immune responses like cytokines and co-stimulatory factors can activate adaptive immune components in B and T cells. Later, various mechanisms trigger an antimicrobial process to combat infection. In this regard, PA as a PAMP affecting TLRs not only provokes innate immune responses but also causes T cell–mediated immunity. One of the most prominent functions of T cells is the activation of macrophages to kill intracellular microbes like the leishmania parasite.[9,10] This protein binds to the T cells via MHC II receptors and induces many immune responses such as lymphocyte proliferation, complement activation, increase of macrophage phagocytosis, and release of cytokines such as IL-1, IL-2, IFN-λ, IFN-α, TNF-α, and IL-8.[11,12] PA can also bind to tumor necrosis factor receptor1 (TNFR1) at the surface of macrophage and respiratory epithelial cells. Thereby, it can activate the downstream signaling pathways of these receptors. It is estimated that PA can stimulate phosphorylation in mitogen-activated protein kinases (MAPKs) and their activation.[13] MAPKs are often involved in pro-inflammatory signaling pathways and stimulate transcription factors such as NF-κB and AP-1 that mediate the production of inflammatory cytokine IL-8.[14] Moreover, upregulating the downstream pathways of TNFR1 receptors increases polymorphonuclear leukocytes' (PMNs) chemotaxis at the site of infection.[15] PA increases the phagocytic index in macrophages, which is attributed to the rise of toxic-free radicals like nitric oxide (NO).[16] According to previous studies, tyrosine kinase and NF-κB signaling pathway contribute to the production of NO following superantigens (e.g., PA)–induced signaling cascade in macrophages. Thus, PA as an inducer of NO production plays an important role in helping macrophages eliminate intracellular parasites.[17] As mentioned previously, findings of past studies reveal consistency between cytokines produced prior to PA intervention and those PA-induced cytokines' response to leishmania infection. According to these potential immunomodulatory properties of PA, we supposed that PA can be a promising candidate to assist immune reactions in the leishmania parasite–infected site. Based on these evidences, the present study explored the potential protective role of PA as a stimulating factor for immune system response in animals infected with the leishmania parasite.

Materials and Methods

Parasites and culture condition

This research was financially supported under Grant No. 398617 by the Isfahan University of Medical Sciences. The Iranian reference strain of Leishmania major (MHRO/IR/75/ER) was provided by the Department of Parasitology at the Isfahan University of Medical Sciences. A frozen vial including parasite was removed from the nitrogen tank. A drop of parasite was examined using a light microscope. After confirming the presence of live cells, the parasite vial was rinsed in RPMI 1640 (Bioidea, USA) under sterile condition in several steps to remove the DMSO effect. It was then transferred to the pre-prepared Novy-MacNeal-Nicolle medium (NNN), a two-phase culture medium. The samples were placed in refrigerator incubator at 24–25°C temperature while the growth of promastigotes was checked every 2 hours. After growing the promastigotes in the liquid phase up to a mass growth in the logarithmic phase for use in animal infection, the parasite was added in RPMI liquid and single-phase culture medium enriched with 20% FCS and antibiotics penicillin G (100 IU/ml) and streptomycin (100 µg/ml). The tubes were re-incubated at 25°C to multiply the parasites. Finally, promastigotes were examined under a microscope and the number of parasite cells was counted by a neobar slide.

Experimental animals

For this stage, 24 three to four-week-old female BALB/c mice were obtained from Isfahan Royan Institute. The animals were kept in favorable conditions in terms of nutrition, room temperatures, and other environmental factors for 2 weeks. All measures related to clinical trial phase were designed in compliance with the guidelines of the ethics committee in medical research at the Isfahan University of Medical Sciences. A total of 10^6 parasite cells were injected into each mouse at the base of the tail. After detecting the skin lesion, the mice were divided into four groups. Each group contained 6 mice. The treatment group received 60 mg/kg body of recombinant PA for 4 weeks and 3 times a week at the base of the tail subcutaneously. The positive control group received Amphotericin B at a dose of 1 mg/kg body weight. The sham group received PA solvent (sterile H2O), and no intervention was performed for the negative control group. The mean size of leishmania lesions was measured during 4 weeks, twice a week, and at the end of the treatment period by a caliper with an accuracy of 0.01 mm. The average wound area calculated in each group has been shown in Table 1.

Table 1: Mean size of the lesion during 4 weeks of treatment. The unit of numbers is Mm^2

	Protein A	Protein A solvent	Without treatment	Amphotericin B
First week	6.87	6.24	8.23	4.98
Second week	6.34	7.14	10.43	5.61
Third week	5.67	6.78	10.87	4.02
Fourth week	5.10	6.50	12.54	4.35

Staphylococcus aureus protein A

Staphylococcus aureus protein A (NCTC 8325. SAOUHSC_00069 [3919448]; Sigma-Aldrich, cat-P6031) was provided in soluble form with concentration of 1 mg/ml.

DNA extraction

At the end of week 4 of the treatment period, spleen DNA was extracted from the mice in all four groups using the DNeasy Blood and Tissue Kits (QIAGEN). The concentration of the extracted samples was determined using a spectrophotometer and DNA Staining Dyes (Green Viewer™) stained 1.5% agarose gel.

Primer design and real time PCR

The specific primers for PCR were designed according to 18s rRNA gene region of leishmania major parasite using Generunner. exe software (V. 6.5.52). [Table 1] The real-time PCR reaction was performed in 20-µL reaction mixture containing 10 µL of Biofact 2X Real-time master mix (Syber Green), 0.7 µL of forward primer (5′-GCCATTTTTGCTTAATGG-3′), 0.7 µL of reverse primer (5′-CTCAGGTCTGTAAACAAAGGTT-3′), 1 µL of DNA, and 6 µL of H2O. The temperature of the protocol was 94°C over 10 minutes, 40 cycles of 30 seconds at 94°C, and 40 seconds at 62°C. The threshold cycle values were determined using StepOnePlus software v 2.3 (Applied Biosystem, USA).

Statistical analysis

The date collected from real-time PCR reactions were statistically analyzed with SPSS (version 23). And the data extracted from the measurement of lesion size were statistically analyzed with GraphPad Prism version 9.2.0.332 (GraphPad Software, Inc., San Diego, CA). Our data did not show normal distribution; thus, we utilized the nonparametric Mann–Whitney *U* test to estimate the post-treatment differences between the groups.

Results

The Leishmania skin lesions on the mice

Measurement of lesion dimensions after treatment and comparison with other group showed that mean wound size area did not decrease remarkably and small lesions did not disappear after treatment with PA. Lesion size differences between positive and negative control were statistically significant ($p \leq 0.05$), but comparing the results between PA treatment group and negative control group did not reveal a significant difference ($p = 0.1$). Therapeutic effect of the treatment of leishmania major–infected BALB/c mice with PA on the size of the lesion has been shown in Figure 1.

The assessment of 18s rRNA gene copy number

After real-time PCR reaction, the Ct results were compared in the intervention and control groups. The number of Ct related to the parasite was obtained using StepOnePlus software (version 3.2). Considering the difference in the Ct values between the four groups, parasite burden in the negative control group was 2^5, 2^9, and 2^{14} times more than ddH_2O, PA, and positive control intervention groups, respectively. The evaluation of fold-change values for the 18s rRNA gene expression in PA treatment and negative control did not represent a significant result ($p = 0.18$), but significant differences in the expression level of 18s rRNA gene were found between Amphotericin B and negative control groups ($p = 0.03$).

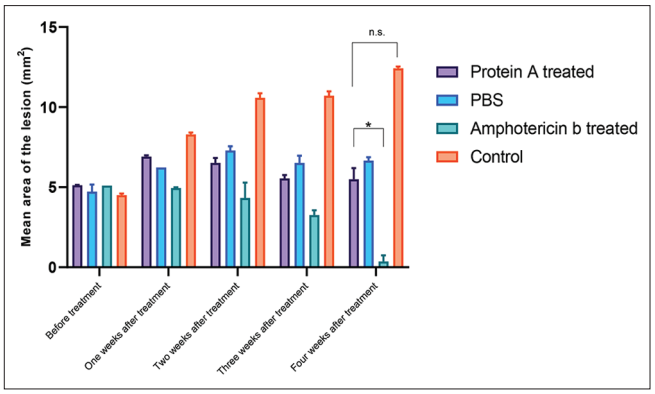

Figure 1: Therapeutic effect of the treatment of Leishmania major infected BALB/c mice with PA on the size of the lesion. Error bars indicates standard deviation in triplicate repetition. n.s = non-significant, * = ($P \leq 0/05$)

The comparative chart of the fold change of the 18s rRNA gene expression in leishmania major–infected mice after treatment period has been shown in the Figure 2.

Discussion

In the present study, the effects of PA on the severity of infection in the infected Balb/c mice were evaluated. Wound size is the most clinical manifestation of cutaneous leishmaniasis.[18] The results demonstrated that PA may play a role in reducing inflammation in the affected skin area. The dose of PA used in this study is based on the previous study which had tested the effect of PA on *Leishmania donovani*.[19] Moreover, an examination on 18s rRNA gene copy number provided a similar result. There is also a lot of evidence available on the involvement of PA in the production pathways of the inflammatory cytokines.[9,11,13] Such a result could be justified with the effect of PA in enhancing the ability of macrophage phagocytosis, which is supposed the most effective response against leishmaniasis infection.[16] The most effective anti-leishmania response of macrophages is the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[20-22] The two main producers of these species are NADPH oxidase 2 (NOX2) and induced nitric oxide synthase (iNOS), which create superoxidase (O2) and nitric oxide (NO), respectively.[23] Many cytokines are produced in response to PA, such as IL-1, IL-2, IFN-λ, IFN-α, TNF-α, and IL-8. These cytokines, in turn, may bind to upstream elements of the iNOS gene promoter and stimulate the transcription of this gene.[24] The results of previous studies show evidence that tyrosine kinase, phospholipase C, and protein kinase C (PKC) participate in the protein-induced signaling cascade

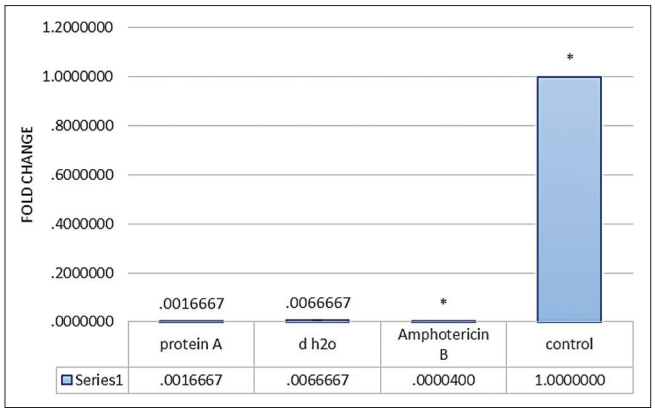

Figure 2: The comparison of the fold change between Leishmania major infected mice after treatment period. *=($P \leq 0/05$)

in macrophages and lymphocytes of the rat spleen to produce NO.[25] Moreover, the increased tyrosine kinase activity with IFN-λ cytokine can increase the iNOS gene expression.[26] Meanwhile, the treatment with tyrosine kinase inhibitors has an inhibitory effect on NO production.[27] On the other hand, NF-κB as a transcription factor can increase the iNOS gene transcription and NO production after activation by PA and with the help of PKC activity.[28] In some studies, PA has been used to treat cancer in humans and animal models in which acceptable results have been obtained. The antitumor properties of PA are likely attributed to its ability to activate macrophages and killer cells mediated by lymphokines.[29,30] Therefore, in addition to being an antitumor agent, PA can be considered as a powerful factor against intracellular microbial infections. In one study on the therapeutic and preventive effects of PA against visceral leishmaniosis (VL), it was revealed that the anti-leishmaniosis properties of this protein are due to the increased macrophages-induced phagocytosis and parasite destruction.[19] The findings are consistent with the results of previous studies in which the anti-leishmaniasis properties of PA have been attributed to higher IFN-γ production and other cytokines activating the process of macrophage phagocytosis.[31] Although an antiparasitic role has been suggested for PA in these studies, there are several studies to support the immunosuppressive effect of PA based on its effects on B lymphocytes and antibodies.[32]

Thus, in contrast to the effect of PA as a superantigen and its subsequent effects on phagocytes to produce different cytokines and formations of acute inflammation, its suppressive effect on certain opsonins like antibodies cannot be ignored. As it is known, phagocytosis of different antigens like PA occurs through recognition by specific receptors on the surface of phagocytes like macrophages and neutrophils. These receptors include TLRs and high-affinity receptors for the Fc region of antibodies. After the internalization of the microbe, the antigens according to their nature are attached to different antibodies like IgG to induce phagocytosis. Then IgG's interaction with Fcλ receptor (FcλR) on the surface of phagocytes initiates phagocytosis. The microbicide function of phagocytes and production of different cytokines and chemokines are events induced subsequently through the proteolysis of antigens inside the cell. Previous studies indicate that PA can block this process through binding to the Fc portion of IgG and subsequently eliminate Fc's with FcλR. Therefore, PA prevents phagocytic activation and consequently inhibits the production of inflammatory cytokines by blocking the Fc domain of IgG.[33] Also, the analysis demonstrated that mice deficient in FcλR on the surface of DCs have more susceptibility to CL with increased wound size and parasitic load. Furthermore, there is an apparent relationship between effective immune system response and FcλR function in DCs in CL.[34] A number of experiments showed that formation of antibody complex through their Fc regions' interaction with PA can prevent the activation of complement system. Complement factor C3 has a crucial role in the opsonization of antigens and phagocytosis procedure. Therefore, the present study suggests an additional immunosuppressive roll for PA.[35]

As mentioned previously, many cytokines are produced in response to PA which acts as a ligand for TLRs on macrophages and DCs and trigger innate immune response. Interaction between PA and TLRs provokes the activation of different protein kinase and signaling pathways like NF-κB and AP-1. Activation of the NF-κB signaling pathway leads to the expression of the *TNF* gene, acute inflammation, and release of cytokines. The evaluation of TNF level's effect on the sternness of CL infection has shown that there is a strong relationship between a higher level of TNF production and increased wound size and intensity of lesions. Our findings support the effects discussed in the foregoing and suggest that applying the inhibitory factors for TNF can a potential therapeutic option to control the ulcer severity.[36]

A simple explanation for the various results obtained in this study in light of the findings of another study that measured the effect of the PA on the VL infection is the differences in cytokine milieu and their functions in controlling the infections caused by L. major and L. donovani. For instance, the overproduction of IL-4 after the internalization of the parasite in the CL is associated with enhanced susceptibility to the infection through polyamine biosynthesis. But it seems likely that there is no significant role for IL-4 in the progression of the VL infection.[37] Generally in VL, the Th1 immune response has a protective role, but Th2 immune responses cause more susceptibility to the progression of infection. The highly significant level of IL-10 in VL suppresses the production and activation of NO and TNF-α; thus, PA neutralizes the immunosuppressive effect of IL-10. Also, treatment with PA causes a considerable increase in IFN-λ production that has an inhibitory effect on IL-10 function. Whereas overexpression of IL-10 in CL is not detected and VL and Th2 responses in CL are also negligible.[38,39]

Since the most of the immune responses against leishmania are limited to the intracellular level and leishmania are intracellular parasites, the antiparasitic effect of B-cell

immunity is insignificant. Findings indicate that B-cell activation in response to the VL can aggravate the infection.[40] This correlation is not yet clear in the case of CL. The total decrease in murine B cells activation and an increase in the development of apoptosis in these cells after treatment with PA can justify the PA's restraining effect on disease expansion in VL infection. This phenomenon is due to PA's ability to bind to the FAB region of antibodies and B-cell receptors.[41] Diversity in gene products can affect the rate of intensity and responsiveness of the immune system to the disease. For example, it has been shown that *SLC11a/NRAMP* gene polymorphism in mice leads to altered sensitivity to the VL infection. Therefore, variation in genetics and environmental factors implicated in the severity rate of different types of leishmania infections should be considered.[42]

Even though we have seen the PA's ability to decrease the wound size in our study, we believe attributing our nonsignificant statistical results supporting PA's ability to decrease the wound size and CL infection would be somewhat premature at this time and more work is needed to gain further insights into PA's functioning as an inhibitor.

According to the present study, PA showed a reducing effect on the wound size but had no significant effect on stimulating the immune system against leishmania.

Conclusion

The results showed that Staphylococcal PA at a dose of 60 mg/kg can decrease wound spread and growth but cannot promote or induce the recovery of leishmania infection on a statistically significant level. Applying different doses of protein may lead to clearer results establishing it as an enhancer of immune response. The findings suggest that protein A is not a good candidate for lishmania treatment but may be suitable in combination therapies with other drugs to speed up the healing process of leishmaniosis infection, a conclusion that needs further confirmation and strengthening with more evaluations in future studies.

Financial support and sponsorship

Nil.

This work was financialy supported by Isfahan University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

References

1. Global leishmaniasis surveillance: 2019–2020, a baseline for the 2030 roadmap. Available from: https://www.who.int/publications-detail-redirect/who-wer9635-401-419. [Last accessed on 2022 Jan 31].
2. Inceboz T. Epidemiology and Ecology of Leishmaniasis. Current Topics in Neglected Tropical Diseases. IntechOpen; 2019. Available from: https://www.intechopen.com/chapters/67175. [Last accessed on 2022 Jan 31].
3. Gholami E, Oliveira F, Taheri T, Seyed N, Gharibzadeh S, Gholami N, *et al*. DNA plasmid coding for Phlebotomus sergenti salivary protein PsSP9, a member of the SP15 family of proteins, protects against Leishmania tropica. PLoS Negl Trop Dis 2019;13:e0007067.
4. Aribi M. Macrophage bactericidal assays. Methods Mol Biol 2018;1784:135-49.
5. Thomas DC. The phagocyte respiratory burst: Historical perspectives and recent advances. Immunol Lett 2017;192:88-96.
6. Votintseva AA, Fung R, Miller RR, Knox K, Godwin H, Wyllie DH, *et al*. Prevalence of *Staphylococcus aureus* protein A (spa) mutants in the community and hospitals in Oxfordshire. BMC Microbiol 2014;14:63.
7. Rigi G, Ghaedmohammadi S, Ahmadian G. A comprehensive review on staphylococcal protein A (SpA): Its production and applications. Biotechnol Appl Biochem 2019;66:454-64.
8. Mazigi O, Schofield P, Langley DB, Christ D. Protein A superantigen: Structure, engineering and molecular basis of antibody recognition. Vol. 32, Protein Engineering, Design and Selection. Oxford University Press; 2019. p. 359-66.
9. Zecconi A, Scali F. *Staphylococcus aureus* virulence factors in evasion from innate immune defenses in human and animal diseases. Immunol Lett 2013;150:12-22.
10. Cellular and Molecular Immunology-9th Edition. Available from: https://www.elsevier.com/books/cellular-and-molecular-immunology/abbas/978-0-323-47978-3. [Last accessed on 2021 Sep 21].
11. Pidwill GR, Gibson JF, Cole J, Renshaw SA, Foster SJ. The Role of Macrophages in *Staphylococcus aureus* Infection. Front Immunol 2021;11:620339.
12. Bröker BM, Mrochen D, Péton V. The T cell response to staphylococcus aureus. Pathogens 2016;5:31.
13. Gómez MI, Lee A, Reddy B, Muir A, Soong G, Pitt A, *et al*. *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. Nat Med 2004;10:842-8.
14. Xiao K, Liu C, Tu Z, Xu Q, Chen S, Zhang Y, *et al*. Activation of the NF-κ B and MAPK signaling pathways contributes to the inflammatory responses, but not cell injury, in IPEC-1 cells challenged with hydrogen peroxide. Oxid Med Cell Longev 2020;2020:5803639.
15. Dri P, Gasparini C, Menegazzi R, Cramer R, Albéri L, Presani G, *et al*. TNF-induced shedding of TNF receptors in human polymorphonuclear leukocytes: Role of the 55-kDa TNF receptor and involvement of a membrane-bound and non-matrix metalloproteinase. J Immunol 2000;165:2165-72.
16. Mishra A, Dwivedi PD, Verma AS, Ray PK. Mechanism of enhanced phagocytic response in protein a treated rat macrophages. Immunol Lett 1992;34:289-95.
17. Krakauer T, Pradhan K, Stiles BG. Staphylococcal superantigens spark host-mediated danger signals. Front Immunol 2016;7:23.
18. Leishmaniasis Clinical Presentation: History, Physical Examination. Available from: https://emedicine.medscape.com/article/220298-clinical. [Last accessed on 2020 Dec 04].
19. Ghose AC, Mookerjee A, Sengupta K, Ghosh AK, Dasgupta S, Ray PK. Therapeutic and prophylactic uses of Protein A in the control of Leishmania donovani infection in experimental animals. Immunol Lett 1999;65:175-81.
20. Carneiro PP, Conceição J, Macedo M, Magalhães V, Carvalho EM, Bacellar O. The role of nitric oxide and reactive oxygen species in the killing of leishmania braziliensis by monocytes from patients with cutaneous leishmaniasis. PLoS One 2016;11:e0148084.
21. Horta MF, Mendes BP, Roma EH, Noronha FSM, Macêdo JP, Oliveira LS, *et al*. Reactive oxygen species and nitric oxide in cutaneous leishmaniasis. J Parasitol Res 2012;2012:203818.
22. Roma EH, Macedo JP, Goes GR, Gonçalves JL, Castro W de, Cisalpino D, *et al*. Impact of reactive oxygen species (ROS) on the control of parasite loads and inflammation in Leishmania amazonensis infection. Parasit Vectors 2016;9:193.
23. Panday A, Sahoo MK, Osorio D, Batra S. NADPH oxidases: An overview from structure to innate immunity-associated pathologies. Cell Mol Immunol 2015;12:5-23.
24. Muñoz-Carrillo JL, Contreras-Cordero JF, Gutiérrez-Coronado O, Villalobos-Gutiérrez PT, Ramos-Gracia LG, Hernández-Reyes VE. Cytokine Profiling Plays a Crucial Role in Activating Immune System

to Clear Infectious Pathogens. Immune Response Activation and Immunomodulation. IntechOpen; 2018.
25. Zhou X, Yang W, Li J. Ca2+- and protein kinase C-dependent signaling pathway for nuclear factor-kappa B activation, inducible nitric-oxide synthase expression, and tumor necrosis factor-alpha production in lipopolysaccharide-stimulated rat peritoneal macrophages. J Biol Chem 2006;281:31337-47.
26. Cosentino-Gomes D, Rocco-Machado N, Meyer-Fernandes JR. Cell signaling through protein kinase C oxidation and activation. Int J Mol Sci 2012;13:10697-721.
27. Yahfoufi N, Alsadi N, Jambi M, Matar C. The Immunomodulatory and anti-inflammatory role of polyphenols. Nutrients 2018;10:E1618.
28. Morris KR, Lutz RD, Choi HS, Kamitani T, Chmura K, Chan ED. Role of the NF-kappaB signaling pathway and kappa B cis-regulatory elements on the IRF-1 and iNOS promoter regions in mycobacterial lipoarabinomannan induction of nitric oxide. Infect Immun 2003;71:1442-52.
29. Sinha P, Ray PK. Functional dichotomy of a 20-mer and 16-mer peptide derived from *Staphylococcus aureus* protein A: Importance of amino acid sequence. Immunopharmacol Immunotoxicol 2002;24:199-210.
30. Agarwal DK, Ray PK. *In vitro* anti-oxidant property of protein-A of Staphylococcus aureus. Indian J Exp Biol 1991;29:1130-3.
31. Catalona WJ, Ratliff TL, McCool RE. γ Interferon induced by S. aureus protein A augments natural killing and ADCC. Nature 1981;291:77-9.
32. Kobayashi SD, DeLeo FR. *Staphylococcus aureus* protein A promotes immune suppression. mBio 2013;4:e00764-13.
33. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. Nat Rev Microbiol 2015;13:529-43.
34. von Stebut E, Tenzer S. Cutaneous leishmaniasis: Distinct functions of dendritic cells and macrophages in the interaction of the host immune system with Leishmania major. Int J Med Microbiol 2018;308:206-14.
35. Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation | PNAS. Available from: https://www.pnas.org/content/118/7/e2016772118. [Last accessed on 2021 Sep 21].
36. Oliveira F, Bafica A, Rosato AB, Favali CBF, Costa JM, Cafe V, *et al*. Lesion size correlates with Leishmania antigen-stimulated TNF-levels in human cutaneous leishmaniasis. Am J Trop Med Hyg 2011;85:70-3.
37. Elmahallawy EK, Alkhaldi AAM, Saleh AA. Host immune response against leishmaniasis and parasite persistence strategies: A review and assessment of recent research. Biomed Pharmacother 2021;139:111671.
38. Host immune response against leishmaniasis and parasite persistence strategies: A review and assessment of recent research-ScienceDirect. Available from: https://www.sciencedirect.com/science/article/pii/S0753332221004534. [Last accessed on 2021 Sep 21].
39. Khadem F, Uzonna JE. Immunity to visceral leishmaniasis: Implications for immunotherapy. Future Microbiol 2014;9:901-15.
40. Smelt SC, Cotterell SE, Engwerda CR, Kaye PM. B cell-deficient mice are highly resistant to Leishmania donovani infection, but develop neutrophil-mediated tissue pathology. Jornal of Immunology 2000;164:3681-8.
41. Fox PG, Schiavetti F, Rappuoli R, McLoughlin RM, Bagnoli F. Staphylococcal protein a induces leukocyte necrosis by complexing with human immunoglobulins. mBio 2021;12:e0089921.
42. Blackwell JM, Fakiola M, Singh OP. Genetics, transcriptomics and meta-taxonomics in visceral leishmaniasis. Front Cell Infect Microbiol 2020;10:590888.