REVIEW ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

Leishmanial Excretory-Secretory proteins: A potent vaccine candidate

Jalaj Kumar Gour[1*], Manoj Kumar Singh[2]

[1]Department of Biochemistry, Faculty of Science, University of Allahabad, Allahabad-211002, Uttar Pradesh, India;

[2]ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana, India

Manuscript received 16th Dec, 2016, revised 15th Jan, 2017, accepted 06th Feb, 2017

Abstract

Leishmaniasis is caused by the intracellular parasite of the genus Leishmania. It now becomes a major public health problem in many countries all over the world. It is always better to prevent the disease than to treat it. The vaccine prevents disease in the people who receive them and protect those who come into contact with an unvaccinated individual. Because of the large genome and complex biology, developing a vaccine for this pathogen has proved to be a challenging task. Leishmania promastigotes are successfully cultivated incompletely defined medium and their excretory-secretory proteins/factors which may act as antigens are easily purified form culture supernatant of cultured Leishmania species. These leishmanial excretory-secretory antigens serve as a candidate for vaccine development in formulation with muramyl dipeptide (MDP) as an adjuvant. However, currently, there is not a single vaccine is available against any form of leishmaniasis for general human use. According to the estimate of the World Health Organization (WHO), 90% of visceral leishmaniasis (VL) occurs in just five countries (Bangladesh, Brazil, India, Nepal, and Sudan). Those in need are amongst the poorest people in these countries to develop a vaccine. The main purpose of this review is to present only the use of Leishmania excretory-secretory antigens (LESAs) as a candidate for the formulation of a potent vaccine against the severe disease leishmaniasis.

Keywords*:* Excretory-secretory antigens, exosomes, *Leishmania*, vaccine

1. Introduction

The parasite causing the disease leishmaniasis is a single cell microorganism belongs to the genus *Leishmania*. It is an obligatory intracellular pathogen that causes the varied group of infectious diseases leishmaniasis and is capable of infecting wide range of animals including humans**.** This disease has the varied clinical signs and symptoms which includes self-healing cutaneous ulcers to asymptomatic infections, enervating mucocutaneous pathologies to potentially deadly visceral forms. These clinical symptoms are mainly depends on the type of *Leishmania* species involved and also on the immune status of the host[1]**.** The *Leishmania* completes its life cycle as a digenetic manner and it needs two different hosts for its survival and completes its whole life cycle. The parasite exists as a motile, flagellated promastigotes form within the midgut of the insect vector sand fly (Primary, invertebrate host) and as a non-motile amastigotes form within the macrophages of the mammalian host where it survives and replicate inside the phagolysosomes[2]**.**

Corresponding author
Full Address : Department of Biochemistry, University of Allahabad, Allahaba– 211002, INDIA
Phone no. +91 9026169035
E-mail: jalaj19biochem@gmail.com

It is estimated that about 12 million people are affected by the disease leishmaniasis worldwide, and about 2 million new cases are believed to occur annually. It is mainly found in tropical and sub-tropical countries and the disease is prevalent in about 98 countries the worldwide where approximately 350 million people live. However, there is a gross under reporting of the cases from endemic regions and these figures may go up[3]**.** Around 5, 00,000 new cases of fatal visceral leishmaniasis and 1.5 million fresh cases of cutaneous leishmaniasis and are assessed every year. The countries which accounts for approximately 90% cases of the deadly visceral leishmaniais alone are Bangladesh, Brazil, Nepal, India and Sudan, on the other hand Brazil, Afghanistan, Iran, Peru, Saudi Arabia, and Syria accounts 90% cases of cutaneous leishmaniasis[4]**.** In India, Bihar, Assam, West Bengal, and eastern Uttar Pradesh the most affected regions by visceral leishmaniasis, where resistance and relapse against the present anti-leishmanial drugs are on lift up. The present situation of the leishmaniasis become more serious global health threat due to the involvement of two infections i.e. *Leishmania* co-infection with HIV-AIDS. Leishmaniasis infected individuals become immunocompromised and it elevates the chance of other infections and thereby promoting HIV replication and resulting in earlier onset of AIDS[5]**.** There

is a great need for new efficient antileishmanial drugs and other control measures that reduces the risk of combination of HIV co-infection, expansion of endemic regions and resistance to the available drugs**.** The Progress in controlling the leishmaniasis requires improved appreciation of the biology of the parasite to allow novel treatment strategies to be designed.

There are mainly two pathways to export the extracellular proteins from the cells. The conventional secretory pathway which involves passage of secretory proteins by the endoplasmic reticulum/ Golgi-dependent route by using signal peptide-dependent secretory transport and a number of extracellular proteins has been discovered that does not make use of the signal-peptide dependent transport of proteins across the cell and follows another type of pathway i.e. the unconventional secretory pathway for the translocation of protein which is not fully understood[6-8]**.**

For the survival within the hosts in spite of several drugs against them, the unicellular microorganisms have evolved several approaches to alter their nearby milieu and amend the host protective immune responses in its favor by obstructing the anti-microbial action of host. Various molecules that are secreted by the intracellular parasites are concerned with the parasite survival within macrophages of the infected host[9]**.** The occurrence signs, symptoms and persistence or relapse of the disease leishmaniasis mainly depends on both the kind of organism involved and the immune status of the host as well. Different kind of cytokines that produced by T-cell clones are the main players during the leishmaniasis that decides the immune status of the affected individual. On the basis of type of cytokine production during the disease, two different pathways of the immune system come into play i.e. T-Helper 1/ T-Helper 2 (Th1/Th2) types[10]**.**

To achieve the success in the field of parasitic infectious diseases many researcher are working on several molecules which are important for the survival of parasites or targeted by host-specific antibodies or host immune responses. It has been concluded from earlier studies that excreted/secreted (ES) molecules from several intracellular pathogens such as Mycobacterium tuberculosis and Toxoplasma gondii etc. that these molecules which are highly immunogenic in nature and have protective properties to be used as models for vaccine designing[11-13]. In addition, previous studies on trypanosomatid secreted factors indicate that these molecules are involved in eliciting strong immunity and defense against related infection[14]**.** In the same way, is has been seen that culture filtrate proteins of leishmanial promastigotes also elevate the state of protection and strong immunity in L. major-infected BALB/c mice[15]**.** So, it is concluded that the ES antigens of *Leishmania* parasites may generate the protective cellular immune response. Although there is an urgent need of an effective vaccine against the neglected disease leishmaniasis and several aspects of ES proteins are not yet known.

2. Drug For Leishmaniasis

The control measures for the effective cure of the disease leishmaniasis are mainly depends on the chemotherapy and the control of the vector sandfly. The first line of durg i.e. pentavalent antimony compounds such as sodium stibogluconate and meglumine antimoniate are now not in routine use due to the emergence of parasite resistance against them. Another problem with these drugs is their high toxicity, high cost, severe side effects, parental administration route etc[16]. Pentamidine and amphotericin B (AmB), the second line of drug also have serious side effects, high toxicity. Now, the various formulations of the AmB is available which have much low level of toxicity and more potent in action against visceral leishmaniasis but their high cost restricted its use among poor countries. The other drugs like sitamaquine and paromomycin are treatment options but it is under phase trial. The only single effective oral drug miltefosine is currently available for the treatment of leishmaniasis but unfortunately, the same problem with this drug is having the high toxicity and severe side effects such as diahhroea, nausea, vomiting etc[17-18]. Therefore, the advancement in the field of drug alternatives for the effective and safe treatment of disease leishmaniasis is still awaited.

3. Leishmanial excretory- secretary proteins/factors

Leishmania promastigotes were found to release soluble excretory-secretory antigens/factors in the suitable culture medium in vitro. These excretory/secretory proteins/factors have been shown to be antigenic as they react with anti-serum raised against homologous promastigotes**.** However, their biochemical nature is not exactly known. Materials or factors antigenically similar to excretory-factors (EF) are also to be present in the parasite homogenate. In recent years much attention has been focused on the excretory factor (EF) due to their unusual biochemical and immunological properties[19-21].

The most common pathway of protein secretion to transport the secretory factors across the membrane is governed by classical pathway of protein secretion. This type of protein transport route includes endoplasmic reticulum/golgi apparatus (ER/ Golgi apparatus) dependent pathway and the protein contains the N-terminal signal peptides to direct the transport of protein[6]**.** There are some other pathways to transport the secretory proteins that also include pathogenic factors secreted by pathogens[7]. These specialized secretory pathways are known as non-classical/unconventional pathway of protein secretion which is ER/Golgi apparatus independent pathway**.** *Leishmania* uses the same way of protein secretion that lacks the N-terminal signal peptides for transporting virulence factors. This types of protein secretion have no effect of brefeldin A that is used as inhibitor of classical protein secretion[22]**.** It is also reported that the secretion of proteins in *Leishmania* promastigotes is affected by several factors like change in temperature, pH or stress like conditions as in case of metacyclogenesis[23]**.**

The pathogenic parasite *Leishmania* adapted itself to survive within the host cell by combating against the harsh environment inside the host. For the better survival within host adverse environment many virulent factors are excreted or secreted by the parasite to modulate the immune response of the host defense system[9]**.**It is well reported from earlier studies that the unconventional secretory pathway play a very important role in persisting the infection inside the host[7]**.** The study by quantitative analysis of secretory factors or proteins through mass spectrometry discloses the investigation of 151 proteins of *Leishmania donovani* and out of which numbers of proteins are secreted by unconventional secretory pathway[24]**.** A study suggested that HASPB which is expressed non-classically by cell surface of *Leishmania* is responsible for the infection in the host[25] and this route can be employed as potent

drug target in the treatment of leishmaniasis.

It is well studied that the unconventional protein secretion pathway are controlled by posttranslational modifications such as phosphorylation[26], cell differentiation[27-28] and also by NF-κβ dependent signaling pathway[29]. In case of protozoan parasite *Leishmania*, various proteins are secreted by the parasite as virulence factors to survive within the host[30]. It is well documented from experiments that an exosome based secretory system plays a crucial role in the secretion of leishmanial proteins and its transport to host cells[31]. that follows the unconventional mode of protein secretion. A study on *Leishmania donovani* suggested that only 14% of the secretory proteins contained the N-terminal signal peptide that follows classical pathway of protein secretion[24]. In another study, Brefeldin A showed no effect on protein secreted by *Leishmania* that confirms the unconventional route of protein secretion in *Leishmania* i.e. mainly based on the exosome based protein secretion pathway. These exosome are small (30-100nm) organelles and is released by various mammalian cells such as B-cells, T-cells also[22, 31]. The viral or bacterial infected cells, various tumor cells also releases exosomes[32-34]. It is well reported that *Leishmania* uses the exosomes for releasing the secretory proteins that participate in modulating the mammalian immune response by communicating with macrophages[31, 35]. It is also reported that during stress conditions such as elevated temperatures and pH change results in increased release of exosome contining secretory proteins and these proteins are also found the infected macrophages[36, 37]. Therefore, there is urgent need of an improved knowledge on unconventional secretory pathway of protein secretion from *Leishmania* to understand the host parasite interaction and in exploring new drug targets for better control of neglected disease leishmmaniasis.

4. Immune response during leishmaniasis

Among several infectious diseases leishmaniasis is widely popular among various experimental model to understand the generation, maintenance and cause of immune responses failure underlying resistance and susceptibility to infection. It is the major infectious disease having a wide range of clinical manifestations that includes cutaneous, mucocutaneous and visceral forms[38]. The clinical outcomes of *Leishmania* infection depend on the type of infecting species involved and also on the host's immunological response. On experiments with experimental models it has been seen that the most significant role among various immune cell are played by the $CD4^+$ T helper cell which demonstrates that strong IFN-γ production, interleukin (IL)-2 and IL-12 responses is associated with cure of the disease and supports Th1 immune type of immune response that dominates over classical Th2 cytokines or IL-10 response[39, 40].

On infection, high production of antibody takes place with low production of cell mediated immune response. The two main type of immune response during the infection i.e. T-Helper type 1 and T-Helper type 2 comes into play simultaneously which is mainly governed by the secretion of type of cytokines on infection. Th 1 type of response i.e. the production of IL-2, TNF-alpha, IFN- γ, IL-12 mainly helps in the depletion of the disease whereas the Th2 type of cytokine secretion i.e. Il-4, IL-5, IL-10, IL-13 led to the development of disease or helps in survival of parasite in the host[41]. Several experiments have supported the role of these cytokines, such as the role of IFN-γ in depletion of disease was established by working with IFN-γ knockout (KO) mice[42]. In the similar way, the role of other cytokines such as IL-4, IL-13, IL-10 that are responsible for the persistence of the leishmanial infection was expimentally proved by using IL-4 KO BALB/c mice, IL-10 KO mice or IL-4/IL-10 KO mice, IL-4/IL-13 KO BALB/c mice[43]. Finally, production of IFN-γ leads to intracellular death of amastigotes through a common effector mechanism. Macrophage activation is associated with induction of nitric oxide (NO) synthase, which in turn leads to NO-mediated killing. Tumour necrosis factor (TNF)-α is a co-factor with NO[44].

5. Progress in vaccine development for leishmaniasis

The disease leishmaniasis comes under the neglected tropical disease and currently there is no any true antileishmanial drug is available for its proper cure and those which are in use have severe toxicity or side effects. As the vaccine point of view, very little research has been performed against the development of vaccine for the visceral leishmaniasis comparatively with cutaneous leishmaniasis[45]. The visceral leishmaniasis is a mammalian infection which includes dogs, marsupials including humans also. In the, Middle East, Mediterranean basin and several countries of Asian continents the wild canines and domestic dogs are dadly affected by the *Leishmania infantum* while Central and South American dogs are severely affected by *Leishmania chagasi* that have very high rate of infection upto 67% in tropical America[46]. The control of the disease by chemotherapy or eradication of infected dogs is not very effective way. Since, several studies in the field of excretory-secretory (ES) proteins/factors suggested that leishmanial ES antigens have potential to trigger the protective immune response against visceral leishmaniasis[47, 48]. It is also reported that *Leishmania infantum* ES antigens (LiESAp) along with adjuvant MDP capable in inducing strong immunity and complete protection against canine visceral leishmaniais[49] by incuding Th 1 type of immune response. It has been reported that 115-kDa secretory serine protease from *Leishmania donovani* has important role in infection and has potential to become a vaccine candidate[50]. In Situ Immunolocalization and Stage-Dependent Expression of a Secretory Serine Protease in *Leishmania donovani* and Its Role as a Vaccine Candidate).

The main target of formulating a vaccine is to activate the strong immunity against the infecting pathogen. A number of experiments are carried out to achieve such goal such as leishmanization i.e. the use of live parasite to cure the disease by using attenuated, irradiated or non-virulent organisms etc. but in spite of all no potential immunity has been achieved[51, 52].

Another trials for vaccine development includes the use of leishmanial excretory secretory antigens (LESAs) that have shown a positive hope towards the vaccine development against leishmaniasis[48]. The experiments on canine visceral leishmaniasis by using excretory secretory antigens of *Leishmania infantum* and leishmanial recombinant antigens results in potential immune response[47]. In the same manner, the *Leishmania donovani* antigens such Fucose-mannose ligand (FML) in combination with saponin results in protection from zoonotic visceral leishmaniasis upto 92-97%[52]. A recent approach against leishmaniasis is the use of DNA vaccines and it has shown a protective effective role in treating cutaneous leishmaniasis in mice[47], which is mainly capable to induce a

strong Th 1 immune response to achieve protection**.** Further, a potent vaccine for leishmaniasis for humans is still awaited.

4. Conclusion

The international recognition about this disease coordinated by WHO programmes brings renewed optimism that control is feasible, especially in India where 70% of the global burden of VL is found[52]**.** New tools for early identification of cases should facilitate surveillance and enable better co-ordinated control programmes. More widespread control in the poorer regions of the world will only be achievable as the local infrastructure develops to enable delivery of healthcare. The emergence of HIV, drug-resistant strains and changes in the epidemiology of the vector challenge this effort. Long-term control will depend on sustained international effort combined with the necessary resources to develop new tools and ultimately a protective vaccine[53]**.**

A new approach for the development of the vaccine against leishmaniasis is seen which seems to be very much effective in near future to cure or treat the disease leishmaniasis and this new approach rely on the formulation of vaccine through leishmanial excretory secretory antigens (LESAs)[48]**.**

LESAs are the protein/factors which are released in the culture medium of the *Leishmania* parasite. Now, a lot of excretory secretory proteins are identified by several *Leishmania* species culture medium which are used as a strong vaccine candidate preparation[21]**.** These proteins are secreted by the parasite in the phagolysosomal compartment and may serve as an interesting target for the host cellular immune responses. The excretory secretory proteins of the parasite could play a crucial role in the biology or virulence of *Leishmania* parasites that helps the parasite to adapt in the harsh environment of the phagolysosomal compartment of host macrophage. These ES proteins could also be degraded into peptides and further associated with MHC class-II molecules for presenting it to CD4+ T-cell to trigger host cellular immune responses[21, 54]**.**

Moreover, it was recently demonstrated that *Leishmania* specific cytotoxic immune responses are developed by individuals living in areas of *L. major* transmission and play a crucial role in resistance to re-infection. So, it is believe that excretory-secretory (ES) proteins may constitute vaccine candidate and these molecules may constitute interesting new drug targets for the better treatment of the leishmaniasis.

Acknowledgement

The financial support received form University Grant Commission- BSR Research Start Up-Grant [F.30-81/2014 (BSR)], New Delhi, India is greatly acknowledged.

Conflict of interest

The author's declares none.

References

1. Kamhawi, S. Phlebotomine sand flies and *Leishmania* parasites: friends or foes?. *Trends Parasitol*, 22 (2006) 439–445.

2. Desjeux, P. Leishmaniasis: current situation and new perspectives. *Comparative Immunology, Microbiology & Infectious Diseases*, 27 (2004) 305–318.

3. Den Boer M., Argaw D., Jannin J., & Alvar J. Leishmaniasis impact and treatment access. *Clinical Microbiology and Infection*, 17(10) (2011) 1471-7. doi: 10.1111/j.1469-0691.2011.03635.x.

4. Gour, J. K., Srivastava, A., Kumar, V., Bajpai, S., Kumar, H., Mishra, M., & Singh, R. K. Nanomedicine and leishmaniasis: future prospects. *Digest Journal of Nanomaterials and Biostructures*, 4(3) (2009) 495-499.

5. Singh, R.K., Pandey, H.P., Sundar, S. Visceral leishmaniasis (Kala-azar): challenges ahead. *Indian Journal of Medical Research*. 123 (2006)a 331–344.

6. Lee, M. C., Miller, E. A., Goldberg, J., Orci, L., & Schekman, R. Bi-directional protein transport between the ER and Golgi. *Annual Review of Cell and Developmental Biology*, 20 (2004) 87-123.

7. Nickel, W., & Rabouille, C. Mechanisms of regulated unconventional protein secretion. *Nature reviews Molecular cell biology*, 10(2) (2009) 148-155.

8. Rabouille, C. et al. Diversity in unconventional protein secretion. *Journal of Cell Science*, 125 (2012) 5251–5255.

9. Olivier, M., Gregory, D. J., & Forget, G. Subversion mechanisms by which Leishmania parasites can escape the host immune response: a signaling point of view. *Clinical microbiology reviews*, 18(2) (2005) 293-305.

10. Bacellar, O., D'oliveira Jr., A., Jerônimo, S., Carvalho, E.M. IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis. *Cytokine*, 12 (2000) 1228-1231.

11. Maue, A. C., Waters, W. R., Davis, W. C., Palmer, M. V., Minion, F. C., & Estes, D. M. Analysis of immune responses directed toward a recombinant early secretory antigenic target six-kilodalton protein-culture filtrate protein 10 fusion protein in Mycobacterium bovis-infected cattle. *Infection and immunity*, 73(10) (2005) 6659-6667.

12. Saadatnia, G., Ghaffarifar, F., Khalilpour, A., Amerizadeh, A., & Rahmah, N. A Toxoplasma gondii 10 kDa in vitro excretory secretory antigen reactive with human IgM and IgA antibodies. *Tropical Biomedicine*, 28(3) (2011) 606-614.

13.Markikou-Ouni, W., Drini, S., Bahi-Jaber, N., Chenik, M., & Meddeb-Garnaoui, A. Immunomodulatory effects of four Leishmania infantum potentially excreted/secreted proteins on human dendritic cells differentiation and maturation. *PloS one*, 10(11) (2015) e0143063.

14. Mehrani, H., & Mahmoodzadeh, A. Immunological Effects of Leishmania major Secretory and Excretory Products on Cutaneous Leishmaniasis in BALB/c Mice. *Iranian Journal of Parasitology*, 2(2) (2007) 9-19.

15. Tonui, W. K., Mejia, J. S., Hochberg, L., Mbow, M. L., Ryan, J. R., Chan, A. S.,Titus, R. G. Immunization with Leishmania major exogenous antigens protects susceptible BALB/c mice against challenge infection with L. major. *Infection and immunity*, 72(10) (2004) 5654-5661.

16. Croft, S. L., Sundar, S., & Fairlamb, A. H. Drug resistance in leishmaniasis. *Clinical microbiology reviews*, 19(1) (2006) 111-126.

17. Singh, R. K., Srivastava, A., Gour, J. K., & Tiwari, V. K. Targeting Leishmania Species: Nanotechnological Prospects. *Advanced Science Letters*, 5(1) (2012) 11-20.

18. Decuypere, S., Vanaerschot, M., Brunker, K., Imamura, H., Müller, S., Khanal, B., Coombs, G. H. Molecular mechanisms of drug resistance in natural Leishmania populations vary with genetic background. *PLOS Neglected Tropical Diseases*, 6(2) (2012) e1514.

19. Karl, K., Mukerji, K., Kar, S., Sarkar, D., Bhattacharya, A., & Ghosh, D. K. Biochemical and immunological characterization of exometabolites from an Indian strain of Leishmania donovani promastigotes grown in a chemically defined medium. *Molecular and cellular biochemistry*, 108(2) (1991) 157-167.

20. Corrales, R. M., Mathieu-Daude, F., Garcia, D., Brenière, S. F., & Sereno, D. An experimental approach for the identification of conserved secreted proteins in trypanosomatids. *BioMed Research International*, 2010 (2010) 752698. doi: 10.1155/2010/752698.

21. Chenik, M., Lakhal, S., Khalef, N. B., Zribi, L., Louzir, H., & Dellagi, K. Approaches for the identification of potential excreted/ secreted proteins of *Leishmania major* parasites. *Parasitology*, 132 (04) (2006) 493-509.

22. Nickel, W. Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells. *Traffic*, 6(8) (2005) 607-614.

23. Doehl, J. S., Sadlova, J., Aslan, H., Pruzinova, K., Metangmo, S., Votypka, J., Smith, D. F. *Leishmania* HASP and SHERP Genes Are Required for In Vivo Differentiation, Parasite Transmission and Virulence Attenuation in the Host. *PLoS Pathogens*, 13(1) (2017) e1006130.

24. Silverman, J. M., Chan, S. K., Robinson, D. P., Dwyer, D. M., Nandan, D., Foster, L. J., & Reiner, N. E. Proteomic analysis of the secretome of *Leishmania donovani*. *Genome biology*, 9(2) (2008) R35.

25. Stegmayer, C., Kehlenbach, A., Tournaviti, S., Wegehingel, S., Zehe, C., Denny, P., Nickel, W. Direct transport across the plasma membrane of mammalian cells of *Leishmania* HASPB as revealed by a CHO export mutant. *Journal of cell science*, 118(3) (2005) 517-527.

26. Nickel, W. The mystery of nonclassical protein secretion. *The FEBS Journal*, 270(10) (2003) 2109-2119.

27. Cooper, D. N., & Barondes, S. H. Evidence for export of a muscle lectin from cytosol to extracellular matrix and for a novel secretory mechanism. *The Journal of Cell Biology*, 110(5) (1990) 1681-1691.

28. Lutomski, D., Fouillit, M., Bourin, P., Mellottee, D., Denize, N., Pontet, M., Joubert-Caron, R. Externalization and binding of galectin-1 on cell surface of K562 cells upon erythroid differentiation. *Glycobiology*, 7(8) (1997) 1193-1199.

29. Wakisaka, N., Murono, S., Yoshizaki, T., Furukawa, M., & Pagano, J. S. Epstein-Barr virus latent membrane protein 1 induces and causes release of fibroblast growth factor-2. *Cancer research*. 62(21) (2002) 6337-6344.

30. Lambertz, U., Silverman, J. M., Nandan, D., McMaster, W. R., Clos, J., Foster, L. J., & Reiner, N. E. Secreted virulence factors and immune evasion in visceral leishmaniasis. *Journal of leukocyte biology*, 91(6) (2012) 887-899.

31. Silverman, J. M., Clos, J., de'Oliveira, C. C., Shirvani, O., Fang, Y., Wang, C., Reiner, N. E. An exosome-based secretion pathway is responsible for protein export from Leishmania and communication with macrophages. *Journal of Cell Science*, 123(6) (2010) 842-852.

32. Bhatnagar, S., Shinagawa, K., Castellino, F. J., & Schorey, J. S. Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response in vitro and in vivo. *Blood,* 110(9) (2007) 3234-3244.

33. Graner, M. W., Alzate, O., Dechkovskaia, A. M., Keene, J. D., Sampson, J. H., Mitchell, D. A., & Bigner, D. D. Proteomic and immunologic analyses of brain tumor exosomes. *The FASEB Journal*, 23(5) (2009) 1541-1557.

34. Liu, C., Yu, S., Zinn, K., Wang, J., Zhang, L., Jia, Y., Zhang, H. G., Murine mammary carcinoma exosomes promote tumor growth by suppression of NK cell function. *The Journal of Immunology*, 176(3), 2006, 1375-1385.

35. Atayde, V. D., Aslan, H., Townsend, S., Hassani, K., Kamhawi, S., & Olivier, M. Exosome secretion by the parasitic protozoan *Leishmania* within the sand fly midgut. *Cell reports*, 13(5) (2015) 957-967.

36. Silverman, J. M., & Reiner, N. E. *Leishmania* exosomes deliver preemptive strikes to create an environment permissive for early infection. *Frontiers in cellular and infection microbiology*, 1 (2012) 1-8.

37. Silverman, J. M., Clos, J., Horakova, E., Wang, A. Y., Wiesgigl, M., Kelly, I., Reiner, N. E. *Leishmania* exosomes modulate innate and adaptive immune responses through effects on monocytes and dendritic cells. *The Journal of Immunology*, 185(9) (2010) 5011-5022.

38. Saporito, L., Giammanco, G. M., De Grazia, S., & Colomba, C. Visceral leishmaniasis: host–parasite interactions and clinical presentation in the immunocompetent and in the immunocompromised host. *International Journal of Infectious Diseases*, 17(8) (2013) 572-576.

39. Nylen, S., & Gautam, S. Immunological perspectives of leishmaniasis. *Journal of global infectious diseases*. 2(2) (2010) 135-146.

40. Couper, K. N., Blount, D. G., & Riley, E. M. IL-10: the master regulator of immunity to infection. *The Journal of Immunology*, 180(9) (2008) 5771-5777.

41. Roberto Castellano, L., Correia, D., Argiro, L., Dessein, H., Prata, A., Dessein, A., & Rodrigues, V. Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-γ production. *Human immunology*, 70 (6) (2009) 383-390.

42. Murray, H. W., Lu, C. M., Mauze, S., Freeman, S., Moreira, A. L., Kaplan, G., & Coffman, R. L. Interleukin-10 (IL-10) in experimental visceral leishmaniasis and IL-10 receptor blockade as immunotherapy. *Infection and immunity*, 70(11) (2002) 6284-6293.

43. Roberts, M. T., Stober, C. B., McKenzie, A. N., & Blackwell, J. M. Interleukin-4 (IL-4) and IL-10 collude in vaccine failure for novel exacerbatory antigens in murine *Leishmania major* infection. *Infection and immunity*, 73(11) (2005) 7620-7628.

44. Das, L., Datta, N., Bandyopadhyay, S., & Das, P. K. Successful therapy of lethal murine visceral leishmaniasis with cystatin involves up-regulation of nitric oxide and a favorable T cell response. *The Journal of Immunology*, 166(6) (2001) 4020-4028.

45. Kedzierski, L. Leishmaniasis vaccine: where are we today? *Journal of global infectious diseases*, 2(2) (2010) 177-185.

46. Bhargava, P., & Singh, R. Developments in diagnosis and antileishmanial drugs. *Interdisciplinary perspectives on infectious diseases*, 2012 (2012) 1-13.

47. Joshi, S., Rawat, K., Yadav, N. K., Kumar, V., Siddiqi, M. I., & Dube, A. Visceral leishmaniasis: advancements in vaccine development via classical and molecular approaches. *Frontiers in Immunology*, 380 (5) (2014) doi: 10.3389/fimmu.2014.00380.

48. Gour, J. K., Kumar, V., Singh, N., Bajpai, S., Pandey, H. P., & Singh, R. K. Identification of Th1-responsive leishmanial excretory–secretory antigens (LESAs). *Experimental parasitology*, 132(3) (2012) 355-361.

49. Choudhury, R., Das, P., Bhaumik, S. K., De, T., & Chakraborti, T. In situ immunolocalization and stage-dependent expression of a secretory serine protease in *Leishmania donovani* and its role as a vaccine candidate. *Clinical and Vaccine Immunology*, 17(4) (2010) 660-667.

50. Choudhury, R., Das, P., De, T., & Chakraborti, T. 115kDa serine protease confers sustained protection to visceral leishmaniasis caused by Leishmania donovani via IFN-γ induced down-regulation of TNF-α mediated MMP-9 activity. *Immunobiology*, 218(1) (2013) 114-126.

51. Das, A., & Ali, N. Vaccine development against *Leishmania donovani*. *Frontiers in immunology*, 3 (2012) 1-19.

52. Roberts, M. T. M. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. *British Medical Bulletin,* 75(1) (2005) 115-130.

53. Bethony, J. M., Cole, R. N., Guo, X., Kamhawi, S., Lightowlers, M. W., Loukas, A., Hotez, P. J. Vaccines to combat the neglected tropical diseases. *Immunological reviews*, 239(1) (2011) 237-270.

54. Sundar, S., & Singh, B. Identifying vaccine targets for anti-leishmmanial vaccine development. *Expert review of vaccines*, 13(4) (2014) 489-505.