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MANAGEMENT OF KALA-AZAR IN BIHAR: SCOPE FOR VACCINE DEVELOPMENT

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Abstract

Leishmania causes a spectrum of diseases globally ranging from self-healing cutaneous to non-healing mucocutaneous and fatal visceral infection forms. Visceral leishmaniasis (VL), caused by the parasite Leishmania donovani, widely affects resource-poor populations in Bihar, India. Post kala-azar dermal leishmaniasis (PKDL) is a sequel of VL. People with PKDL, whose number is gradually on the rise in India, are considered to be a silent parasite pool for transmission of VL. Despite many options, treatment of VL in India is far from satisfactory due to high incidence of PKDL development, drug-resistance, relapse, drug-toxicity, long-dose regimens, need for parenteral administration and high cost. Currently, there is no approved vaccine for human use in leishmaniasis. However, several leishmaniasis vaccine candidates are at various stages of development. Vaccines such as Leish-F1, F2 and F3, are designed based on selected Leishmania antigenic epitopes, have entered the phases of clinical trials. Other groups, including the Sabin Vaccine Institute in collaboration with the National Institutes of Health are working on development of a recombinant Leishmania antigen vaccine with selected sand fly salivary gland antigens targeting to elevate the host immunity status. The state of Bihar in India is heavily affected by VL accounting for over 90% cases of India. In this review, we will discuss the strategies of Leishmaniasis vaccine development, details of vaccines in the developmental pipeline and about future perpectives of an ideal vaccine for effective management of Kala-azar in Bihar.

Introduction

The *Leishmaniadonovani* complex (comprising *L. donovani*, *L. infantum* and *L. chagasi*), an obligate intracellular protozoan parasite, is responsible for a fatal progressive systemic disease- visceral leishmaniasis (VL), commonly referred to as “kala azar” or “black fever”. Leishmania parasite follows a digenetic lifestyle by remaining either as extracellular and flagellated promastigotes within sand fly or as intracellular and non-flagellated amastigotes within the mammalian host. The parasites mainly affect mononuclear phagocytic cells of the reticuloendothelial organs, viz. liver, spleen, and bone marrow. However, they are usually disseminated to other visceral organs (gut, lung, etc), as well as the skin, particularly seen in *L. infantum*-affected areas [1]. Persistent fever, hepatosplenomegaly, pancytopenia, and hypergammaglobulinemia are the characteristic features of human VL [2]. The case fatality rate of this disease is 100% because the patient dies in the absence of treatment. Available chemotherapeutics have several demerits, and their usage is also limited due to the fact that only ailing individuals with clinical symptoms are benefited.

Human VL occurs in a more heterogeneous form with varied chronicity. Only few infected individuals develop clinical symptoms, and on this basis, they are the ones who are subjected to antileishmanial chemotherapy. However, the majority of the endemic population remains with subclinical infection or as asymptomatic carriers (~6–10 times more than the number of VL patients) and their role in disease transmission is yet to be confirmed [3]. Moreover, the immunocompromised state of VL patients makes them susceptible to other secondary infections.

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Emergence of parasite resistance following drug treatment and increased reporting of disease relapse is another major problem in the process of elimination. Furthermore, because the treatment end point is not defined, as patients often fail to report back 6 months posttreatment, it is difficult to ascertain the complete cure from the disease.

Occasionally, approximately 10%–50% of VL patients, during or after the treatment, develop post kala azar dermal leishmaniasis (PKDL), which is characterized by maculopapular or nodular skin rashes [4]. It is commonly seen in East Africa and the Indian subcontinent and requires prolonged and expensive treatment. The role of PKDL in transmission of VL is debatable. [5](Desjeux et al., 2013).

Epidemiology of VL in India

In India, visceral leishmaniasis (VL) or, Kala-azar is endemic in the four north-eastern states: Bihar, Jahrkhand, Uttar Pradesh and West Bengal. Present spatial spared of VL endemicity limited to 54-districts of these four states. Bihar is epicenter; 33 out of 38 districts are VL affected, of which 10 districts (i.e. Araria, East-Champaran, Gopalganj, Muzaffarpur, Purnia, Saharsa, Siwan, Saran, Sitamrhi and Darbhanga) are highly endemic (report 200 or, more cases annually) and contribute to more than 70% cases of the state [6]. Bihar alone contributes more than 70% of the total Indian cases annually. In 2017, 71.7% of the total Indian cases were reported from the Bihar state only, where as 23.6 % in Jharkhand, 2% in Uttar Pradesh and 2.7% in West Bengal. An approximately 99-million people are at risk in the 1200 villages over the 458 blocks [7]. From 2002 to 2017, a total of 2, 80,780 VL cases with 1,380deaths were reported in Bihar; annul cases ranged from 9505 in 2002 to 4,127 in 2017. Duringthis period,two rising trends were observed (i.e. one in 2002-2007 and another in 2009-2011) [Fig 1]. The highest peak of VL cases occurrence was found in 2007 (i.e. 37, 822). There is a sharp decline in the mortality rate, from 158 in 2002 to 0 in 2017. A decreasing trend was observed in 2012 and 2011 onwards for cases occurrence and mortality, respectively [Fig 1]. Annul case incidences (i.e. 10,000 populations) among the endemic districts varied from 0.01 to 15.44 (mean±SD = 1.64 ±2.66). The cumulative hot-spot map shows the spatio-temporal distribution of VL-endemicity level throughout the state at four years interval between 2002 and 2017 [Fig 2]. A persistence high level VL cases occurrence was observed in the center and north-eastern part of the state between 2002 and 2013. However, in 2014 to 2017 intensity of VL cases occurrence ranged between low and moderate levels throughout the state and it indicates a good progress in the VL elimination programme of Bihar state to achieve the elimination target in the near future. Although the number of VL incidence have been decreased and the mortality rate has been ensured ‘zero’ reporting at all the endemic zones of the state, the increasing trend in the PKDL cases occurrence could be a major concern point for an epidemic outbreak/resurgence of the new VL cases in the future [Fig 3] [8,9]. This will not only disrupt the progress in VL endemicity control in the region but also make the situation difficult to sustain the disease occurrence in the low endemic or, non-endemic regions.

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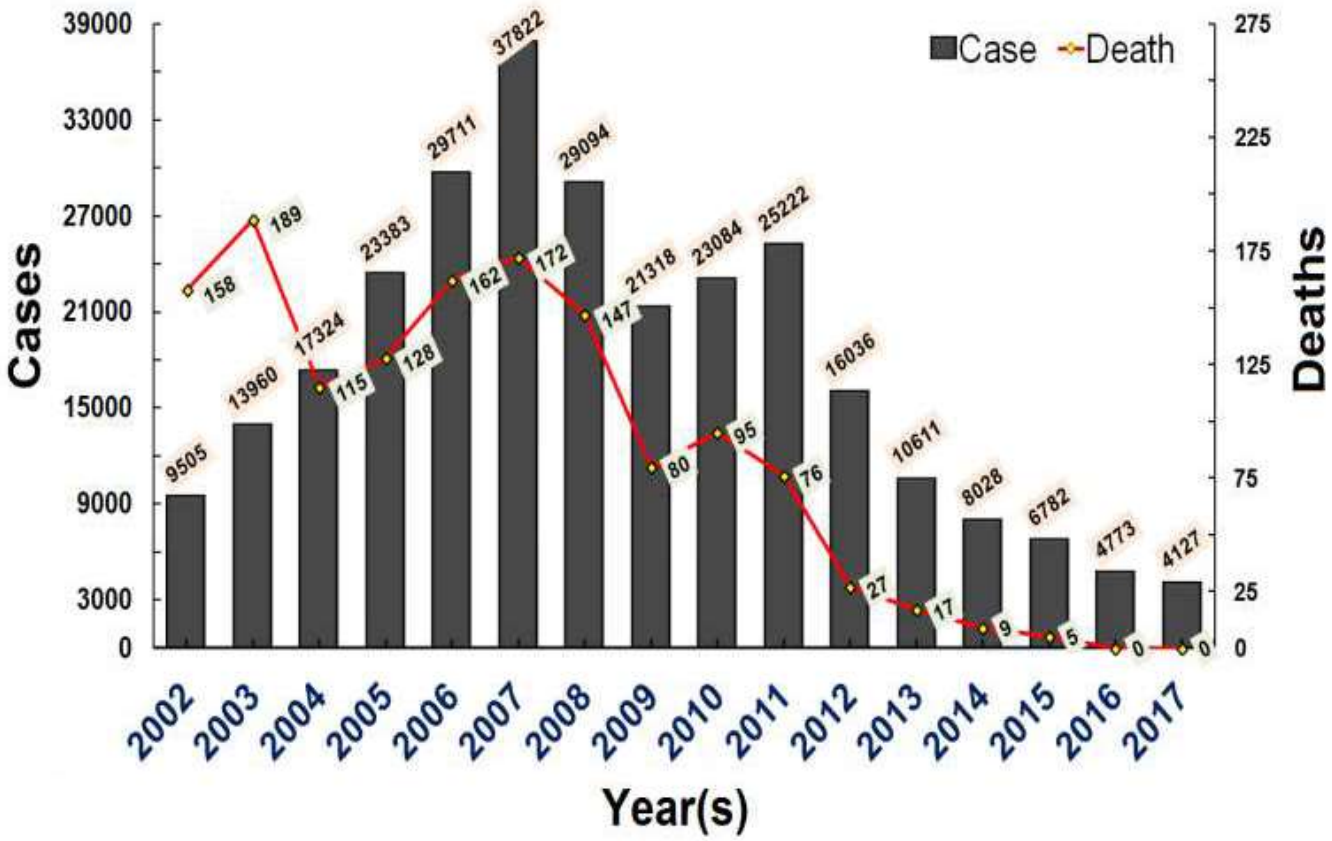

Figure 1: Number of VL cases and deaths occurred in Bihar state between 2002 and 2017.
Data sources: Bihar State Health Society and the District Malaria Office, Sheikhpur, Patna (Bihar, India).

Brief outline of the Immunobiology of VL

The host immunology plays a key role for defining the fate of the disease in VL. Therefore, development of a successful vaccine for VL needs to target the immune system of the host. The major innate immune cells that play a significant role in defense against *Leishmania*are neutrophils, macrophages, and dendritic cells (DCs). When the female sandfly sucks blood meal from the vertebrate hosts, flagellate metacyclic forms of *Leishmania*are delivered along with sandfly salivary ingredients into the skin of the hosts [10]. Initially, the promastigotes are taken up by the neutrophils at the site of infection. Following apoptosis in these infected neutrophils, the released parasites infect neighboring macrophages [11,12]. These macrophages are recruited by the chemotactic properties of the proteophosphoglycans, delivered to the infection site by the vector at the time of its blood meal [13,14]. *Leishmania*parasites are phagocytosed following binding to C3b after which promastigotes get converted to the amastigote form [15]. Infection is established following phagocytosis in macrophages, which is determined by several survival strategies of the parasites, most prominent of which is the modulation and attenuation of immune responses. *Leishmania*parasite suppresses the release of Th1 associated cytokines which in turn restrains DCs to present the parasite-specific antigens to the T cells. Thus preventing the activation of the acquired immunity, the parasite gains survival advantage[16, 17].

The induction of CD4+ Th1 cell responses against parasite antigens is crucial in controlling primary infection, when cytokines such as IFN-γ induce nitric oxide production by activated phagocytic cells able to kill internalized parasites. Concomitantly to the role of CD4+ T cells, CD8+ T cells also contribute to protection against disease, and have an important role in controlling primary infections by increasing the Th1 response through a mechanism

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dependent on IFN-γ production. In contrast, cytokines such as interleukin-4 (IL- 4), interleukin-10 (IL-10), interleukin-13 (IL-13), interleukin-18 (IL-18), and transforming growth factor beta (TGF-β) represent disease promoting molecules which inhibit the Th1 response, contributing to the deactivation of infected macrophages and, consequently, to the development of disease [18]. Although, VL initially was thought to be associated with a Th2-type immune response seen as elevated levels of IL-4 and/or IL-13 [19, 20], some recent studies implicate that there is not a clear Th2 skewing in human VL[21-23]. Typically VL is associated with increased production of multiple and primarily pro-inflammatory, cytokines and chemokines. VL patients have been found to have elevated plasma protein levels of IL-1, IL-6, IL-8, IL-12, IL-15, IFNγ inducible protein-10 (IP-10), monokine induced by IFNγ (MIG), IFNγ, and TNFα [20-22]. An elevated level of IFNγ mRNA has been found in the spleen and bone marrow during the acute phase of infection [23]. These observations suggest that development of VL is not driven by Th2 skewing per se, but that other mechanisms also contribute to the pathogenesis of VL [20-23].

Clinical studies strongly implicate the role of IL-10 in many of the immunologic defects associated with kala-azar [23]. Patients with active VL have elevated levels of IL-10 in serums well as enhanced IL-10 mRNA levels in spleen, lymph nodes, and bone marrow [20, 24]. The main disease-promoting activity of IL-10 in VL is probably conditioning host macrophages for enhanced survival and growth of the parasite. IL-10 can render macrophages unresponsive to activation signals and inhibit killing of amastigotes by down-regulating the production of TNFα and NO [20, 25]. In human VL,inhibition of IL-10 enhance the IFNγ response by antigen-stimulated PBMC, and neutralization of IL-10 in VL serum inhibit *L. donovani*replication in macrophages [20, 25].

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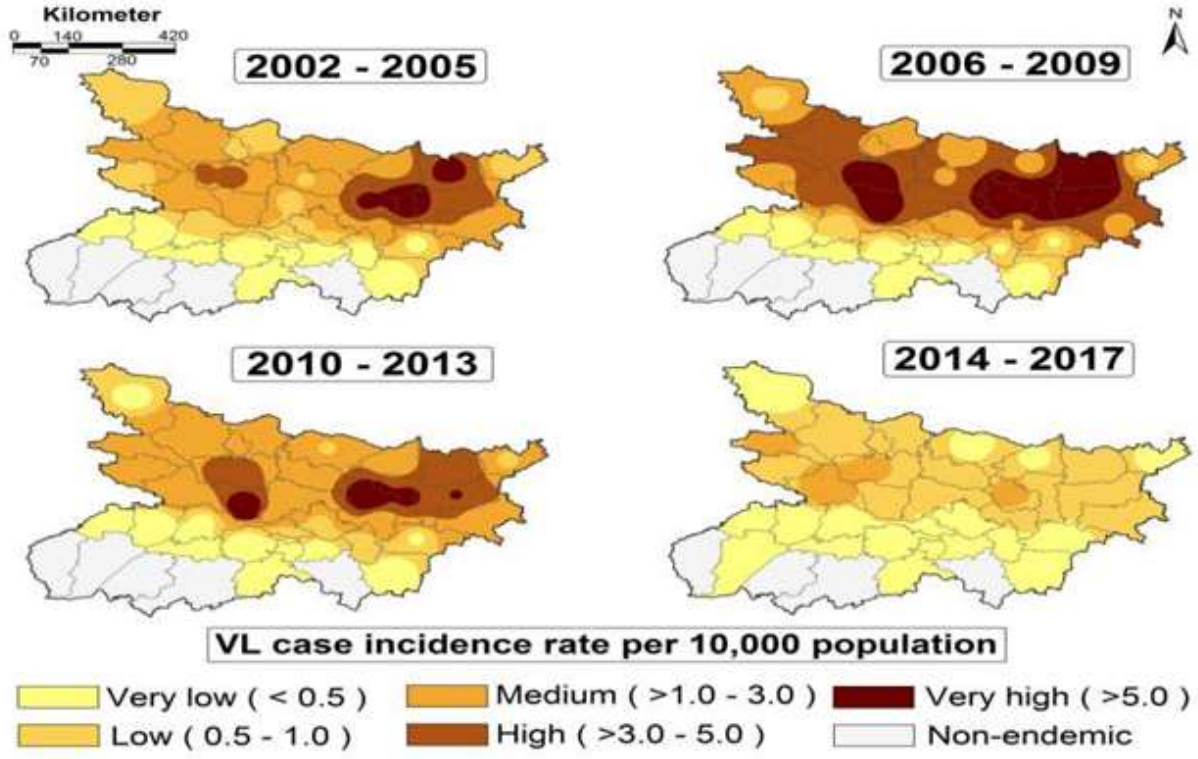

Figure 2: Spatio-temporal distribution of cumulative VL cases occurrence at four years interval from 2002 to 2017. Annual VL incidence rate per 10,000 population calculated at district level and the cumulative spatio-temporal distribution map derived through inverse distance weighted (IDW) interpolation technique in ArcGIS software v9.1 (ESRI Inc., Redlands, CA, USA). Propensity of VL endemicity levels (i.e. low, very low, medium, high and very high) visualizes using the intensity of different colors (i.e. yellow and brown) throughout the Bihar state.
Data sources: Bihar State Health Society and the District Malaria Office, Sheikhpur, Patna (Bihar, India).

Although the classical Th1/Th2 paradigm of resistance/susceptibility appears to be valid during CL, a mixed Th1/Th2 response is required for disease control during VL [26]. However, it is yet to establish a clear Th1/Th2 paradigm for curative and preventive response against both CL and VL. Moreover, for ML, the disease manifestation is largely due to inflammatory response than due to parasite burden. Therefore, conventional Th1/Th2 paradigm does not apply to ML. It has been found that Treg (CD4+CD25+ regulatory T cells) as well as Th17 (other subsets of T cells) cells, play a significant role in disease outcome in both CL and VL, their role in ML is much more complicated. Studies with *Leishmania major* and*L. infantum*have shown a protective role of IL-17 as well as IL-22 (Th17 cytokines) against intracellular parasites [27, 28]. Recently it was shown that when recombinant IL-17 or IL-23 was administered to mice it caused a considerable containment of parasite load in infected organs with significant production of factors such as IFN-γ, nitric oxide, etc. Thus, this study demonstrated the association of Th17-based cytokines in providing protection against the disease [29].

A lower CD4/CD8 ratio has been described in VL PBMC. In human VL a splenic influx of both CD4 and CD8 T cells are observed implicating that CD8 cells may survive better than CD4 cells in VL patients[20], though in the later stage of the disease leukocytes are scanty and plasma cells and macrophages predominate in the spleen [30]. In mouse models of VL, CD8 cells play important role in controlling *L. donovani*/ *L. infantum*infection in liver through their cytolytic activity [31-34]. Moreover CD8 T cell response provide good immune response following

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vaccination.[35](Stager et al., 2000)As an important constituent of the immune system, Treg cells are known to regulate immune response of other cells. These cells were observed to be present in human cutaneous lesions [36]. Increased expression of lesional FoxP3 and IL-10 during progressive *L. major* infection in a murine model and similarly during *Leishmaniabraziliensis*infection in human patients suggest the disease-promoting role of these regulatory cytokines [23]. The preliminary data suggest that despite Th1 polarization production of IL-10 and Treg cells is associated with delayed healing of CL [37]. Apart from T-cell subsets (Treg and Th17) other than conventional T cells, the role of innate immune response has been essentially linked to disease outcome. In fact, engagement of the macrophage toll-like receptors (TLRs) by the parasites has not only shown to improve phagocytosis but also lead to the killing of the parasites due to triggering of NF-κB transactivation and concomitant production of the downstream mediators including pro-inflammatory cytokines. For instance, TLR9 activation has been found to be beneficial for the host against these parasites. But this situation may not be true for all the TLRs. Lipophosphoglycan, a TLR2 agonist has been shown to have antagonizing effect on TLR9 mediated signal cascade in host macrophage, which in turn facilitates parasite survival [38, 39]. Studies showed that treatment with TLR4 and TLR9 agonists decreased the disease severity following challenge infection with *L. major* in BALB/c mice [40]. However, in human VL, comparison of mRNA expression levels between pretreatment and posttreatment splenic aspirate samples showed considerably more TLR2 and TLR4 expression but no change in TLR9 expression during *L. donovani*infection [41]. Furthermore, the later stages of *L. donovani* infection rendered tolerance to macrophages, leading to incapability for the production of inflammatory cytokines like tumor necrosis factor (TNF)-α and interleukin (IL)-1β in response to TLR stimulation.[42]. Despite advances, achieving a comprehensive and clear picture of the immunobiology of leishmaniasis is still required to develop effective interventions such as type-specific vaccine and immunotherapy for leishmaniasis.

Feasibility of suitable Vaccine against Visceral Leishmaniasis (VL)

The World Health Organization, in 2005, has initiated elimination campaigns in VL-endemic regions through early case detection, followed by complete treatment with a target to reduce the number of diseased individuals in the endemic areas. This drive has successfully saved many lives through the administration of antileishmanial drugs. The only control measure for VL presently available is chemotherapy, which includes pentavalent antimonials (meglumineantimoniate and sodium stibogluconate [SSG]), amphotericin B and its liposomal formulation, paromomycin, and miltefosine either alone or in combination [2]. The chemotherapeutics used against VL have been found to be very effective but with several demerits, such as low efficacy, lesser availability, toxicity, high cost of treatment (drugs and hospitalization) and requirement of longer regimens with the invasive route of administration (parenteral) [43]. The achievement of this goal is difficult with chemotherapy alone due to emergence of drug resistance [44]. Furthermore, the presence of asymptomatic individuals in hyperendemicareas might have resulted in disease transmission, thereby creating hurdles for the current VL control program. Hence, there is an urgent need for alternative treatment strategies to control the kala-azar. Over the past few decades, researchers have been trying to develop a vaccine as a cost-effective treatment strategy against VL [45]. It was evident that patients recovered from Kala-azar develop strong immunity, making them resistant to subsequent clinical infections, which indicates that prevention of Kala-azar through either prophylactic or therapeutic vaccination is feasible [46]. Identification of appropriate vaccine candidates through a proper understanding of the immunobiology and pathogenesis of the disease is required for the success of the vaccine development program [41]. Additionally, selection of either a suitable adjuvant or immunomodulatory or an effective delivery system can further boost the immune responses and generate long-lasting immunity [47].

Prophylactic Antileishmanial vaccine

Although initial studies suffered with objectionable side effects resulting into clinical complications, later various leishmanial antigens have been explored as promising vaccine candidate for leishmaniasis. These antigens included killed or live attenuated *Leishmania* parasite (first generation), recombinant Leishmania proteins (second generation), DNA encoding Leishmania proteins (third generation), and immunomodulators[48-51]. Admittedly, at present there is no vaccine available for human use against leishmaniasis.Many scientists are investigating the possibility of vaccination against leishmaniasis[49, 52]. The major challenges in the development of vaccine against leishmaniasis are the complexity associated with the antigenicity, uneven response by the host, variability in the

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different species of the Leishmania, and cost associated with the development [51]. The approaches for prophylactic vaccine studies are summarized in the Table 1.

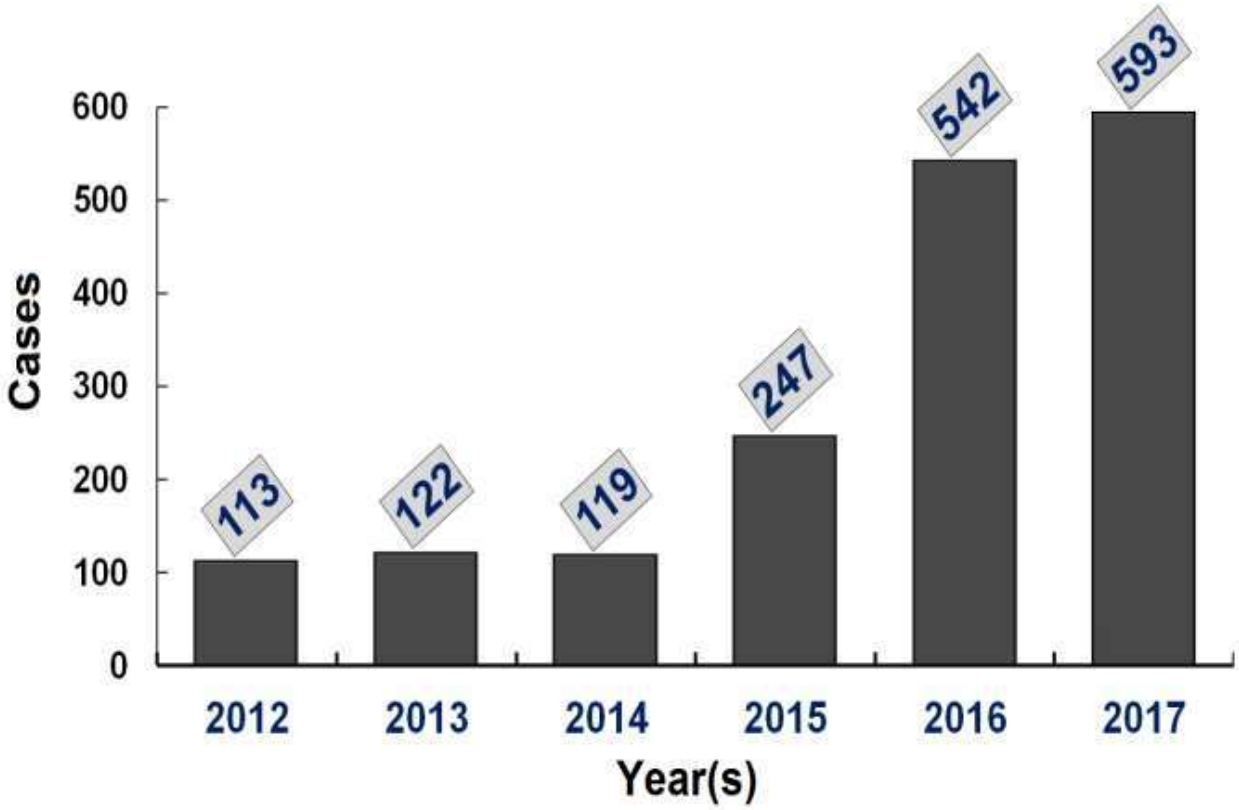

Figure 3: Number of PKDL cases occurred in Bihar state between 2012 and 2017. A 5.2 folds increase observed in occurrence of PKDL cases from 2012 to 2017.
Data sources: Bihar State Health Society and the District Malaria Office, Sheikhpur, Patna (Bihar, India).

Options for Therapeutic Vaccines against VL

Therapeutic vaccines are thought to be essential in case of persistent chronic infections, because in such cases, either intracellular parasites evade the host immune system establishing themselves in a more secured way or the available control intervention is ineffective. In case of VL, the majority of inhabitants in disease affected areas either serves as endemic healthy individual or asymptomatic individual, who serve as the reservoir of parasites or may become symptomatic in future. Hence there is a need for a therapeutic vaccines that can be effectively used to stimulate patients own immune defense system in this endemic population, thereby controlling the progression of the disease.The therapeutic strategy involves the use of biological molecules (whole or their components) in combination with either adjuvants or drugs to modulate the immune responses of *Leishmania*-infected individuals toward the protective type. Therefore, this strategy, which restores or induces an effective immune response without any side effects, could be a promising alternative to conventional chemotherapeutics.The various therapeutic approaches of vaccination against VL according to three generation has been summarized in the following Table 2.

Challenges in vaccine development

The potential approach for the effective control and complete eradication of any infectious disease is vaccination. Over the past two decades, immunotherapy, either alone or in combination with chemotherapy, has been developed

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as an additional approach to combat leishmaniasis. Lifetime immunity against reinfection manifests possibility of developing an effective vaccine (prophylactic and therapeutic) against leishmaniasis.Vaccine development project for leishmaniasis seems to attract less industry people causing a serious crunch of funds.According to the G-Finder, over US$ 66 million has been granted for research and development of vaccine, preventative, and therapeutic, against leishmaniasis largely from chief public sector and charitable trusts (from the year 2007 to 2013). Some of the major funding sources are Carlos Slim Foundation, Bill & Melinda Gates Foundation, Wellcome Trust, Indian Council of Medical Research, European Commission, Institute Pasteur, German Federal Ministry of Education and Research (BMBF), and the U.S. National Institutes of Health (Policy Cures. G-Finder; 2015)[104].*Leishmania*-infected individuals gain considerable lifelong immunity to reinfection, suggesting the feasibility of vaccination. However, regardless of many potential vaccine candidates, translation of these to develop a human administrable antileishmaniasis vaccine is still arduous.There are never ending debates regarding the choice of suitable antigens as vaccine candidate with lower toxicity and greater efficacy and immunogenicity despite of studying a series of antigens as described in Table 1 and 2.*Leishmania*infection follows a complex clinical outcome varying from the cutaneous to visceral form as the parasite is equipped for generating an extensive assortment of atypical and uncommon variations. The virulence factors as well as in the immune responses induced by the different strains and species of *Leishmania*is not fully known. A better understanding of the immunobiology and vaccine (prophylactic and therapeutic) development prerequisites for the different forms of leishmaniasis will provide tools that can be exploited to overcome the virulence dynamics of *Leishmania*species. Preliminary studies are mainly done in mouse and hamster model hence the immune responses leading to protection in humans differs due to lack in correlation to the immune response in the animal model.

The fore-runners in vaccine development

Chemotherapapeutic options are the main treatment for all three major forms of leishmaniasis. However, several factors such as high costs, toxicity, and long-term complicated regimens are hurdles for the chemotherapeutic options. The elimination of leishmaniasis also depends on sand fly vector control approach through indoor residual spraying [IRS] in many areas. Emergence DDT resistancein highly endemic areas has jeopardized IRS leading to large growth of sand flies and potential parasite transmission. Therefore, the development and delivery of an ideal long-term immunity providing vaccine may represent as the most cost effective means of controlling and/or eliminating VL in India.

Ideally, an effective vaccine against leishmaniasis should provide long-lasting immunity and protect broadly against both VL andCL. Many leishmanial antigens have been researched extensively for their use as candidate vaccines in preventative and therapeutic indications; either by delivery as DNA or directly as recombinant proteins. Many such vaccine candidates are at various developmental stages of preclinical testing. The first vaccine candidate that made into phase I and II clinical trials is known as LEISH-F1, developed at The Infectious DiseaseResearch Institute (IDRI, Seattle, WA) [105]. LEISH-F1, is a fusionof three tandem polypeptides in monophosphoryl lipid A-stable emulsion (MPL-SE). Phase I trials showed satisfactory safety and immunogenicity profile in endemic and non-endemic populations of the United States, Colombia, Brazil,Peru and India. Interestingly, LEISH-F1 demonstrated shortened time to cure when used with chemotherapy [106, 107].After the encouraging results of LEISH-F1, IDRIdesigned LEISH-F2 without a histidine tag at the N-terminus, and progressed it into the phase I and a phase II clinical trials. Further changes were made in LEISH-F2 by fusing, in tandem, the open reading frames of nucleoside hydrolase (NH) from *L. donovani* and sterol 24-c-methyltransferase (SMT) from *L. infantum*, with a TL4 based adjuvantglucopyranosyl lipid A to form LEISH-F3. This trial results were promising that found LEISH-F3 to be safe and immunogenic at lower doses.

Other mention-worthy group for vaccine development is the Sabin Vaccine Institute ProductDevelopment Partnership (Sabin PDP). They are exploring prototypecombination vaccines comprised of recombinant proteins encoding sand fly salivary gland antigens and *L. donovani* NH36expressed in either yeast or bacteria. Although there is some encouragingdata from the animal models for this vaccine, the Sabin PDP is still in the R&D phase. Besides this, some other groups of investigators, including the European Multivalent Vaccine for HumanVisceral Leishmaniasis (MuLeVaClin), are working with recombinant protein-based, DNA-based, and heterologous prime-boost vaccine strategies for VL in pre-clinical models [108, 109].

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The epidemiological scenario of VL makes its elimination a realistic goal. Lastly, in the vaccine development scenario, it is very critical to develop an effective vaccine delivery system and adjuvant in order to achieve a successful *Leishmania* vaccine. Besides vaccine development, we should also focus into repurposing of approved drugs and nutraceuticals to be a great new option for treatment of VL. Moreover, increased financing support from major funders will be a critical need to advance candidate vaccine studies for human use in the target population in India.

References

1. *Diro E, Lynen L, Ritmeijer K, Boelaert M, Hailu A, van Griensven J. Visceral leishmaniasis and HIV coinfection in East Africa. PLoSNegl Trop Dis. 2014;8:e2869.*
2. *World Health Organization. 2010. Control of the leishmaniases: Report of a meeting of the WHO Expert Committee on the Control of Leishmaniasis, Geneva, 22–26 March 2010. WHO Technical Report Series, no. 949. WHO, Geneva, Switzerland. Available from: http://apps.who. int/iris/bitstream/10665/44412/1/WHO_TRS_949_eng.pdf. Accessed July 26, 2016.*
3. *Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic Leishmania infection: a new challenge for Leishmania control. Clin Infect Dis. 2014;58:1424-29.*
4. *Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Postkala-azar dermal leishmaniasis. Lancet Infect Dis. 2003;3:87-98.*
5. *Desjeux P, Ghosh RS, Dhalaria P, Strub-Wourgaft N, Zijlstra EE. Report of the post kala-azar dermal leishmaniasis (PKDL) consortium meeting, New Delhi, India, 27–29 June 2012. Parasite Vectors. 2013;6:196.*
6. *NVBDCP. Accelerated plan for kala-azar elimination. National Vector Borne Disease Control Programme.2017. http://nvbdcp.gov.in/Doc/Accelerated-Plan-Kala-azar1-Feb2017.pdf.Accessed 17 Apr 2017.*
7. *Mandal R, Kesari S, Kumar V, Das P. Trends in spatio-temporal dynamics of visceral leishmaniasis cases in a highly-endemic focus of Bihar, India: an investigation based on GIS tools. Parasites &Vectors. 2018;11: 220.*
8. *Ganguly S, Saha P, Chatterjee M, Roy S, Ghosh TK, Guha SK, Kundu PK, Bera DK, Basu N, Maji AK. PKDL-A Silent Parasite Pool for Transmission of Leishmaniasis in Kala-azar Endemic Areas of Malda District, West Bengal, India.PLoSNegl Trop Dis. 2010; 9:10.*
9. *Hirve S, Boelaert M, Matlashewski G, Mondal D, Arana B, Kroeger A, Olliaro P. Transmission Dynamics of Visceral Leishmaniasis in the Indian Subcontinent – A Systematic Literature Review. PLoSNegl Trop Dis. 2016;10:8.*
10. *Khamesipour A. Therapeutic vaccines for leishmaniasis. Expert OpinBiolTher. 2014;14:1641-9*
11. *Mollinedo F, Janssen H, de la Iglesia-Vicente J, Villa-Pulgarin JA, Calafat J. Selective fusion of azurophilic granules with Leishmania-containing phagosomes in human neutrophils. J BiolChem. 2010 285:34528-36.*
12. *Beattie L, Kaye PM. Leishmania-host interactions: what has imaging taught us? Cell Microbiol. 2011;13:1659-67.*
13. *Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P, et al. Proteophosophoglycans regurgitated by Leishmania-infected sand flies target the l-arginine metabolism of host macrophages to promote parasite survival. PLoSPathog. 2009;5:e1000555.*
14. *Rogers ME, Corware K, Muller I, Bates PA.Leishmaniainfantumproteophosphoglycans regurgitated by the bite of its natural sand fly vector, Lutzomyialongipalpis, promote parasite establishment in mouse skin and skin-distant tissues. Microbes Infect. 2010;12:875–9.*
15. *Ueno N, Wilson ME. Receptor-mediated phagocytosis of Leishmania: implications for intracellular survival. Trends Parasitol. 2012;28:335–44.*
16. *Liu D, Uzonna JE. The early interaction of Leishmania with macrophages and dendritic cells and its influence on the host immune response. Front Cell Infect Microbiol. 2012;2:83.*
17. *Kedzierski L. Leishmaniasis vaccine: where are we today? J Glob Infect Dis. 2010;2:177-85.*

September 2018;5(9) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1437512 Impact Factor: 4.054

18. *Duarte MC, Lage DP, Martins VT, Chávez-Fumagalli MA, Roatt BM, Menezes-Souza D, Goulart LR, Soto M, Tavares CA, Coelho EA.Recent updates and perspectives on approaches for the development of vaccines against visceral leishmaniasis.Rev Soc Bras Med Trop. 2016;49:398-407.*
19. *Sundar S, Reed SG, Sharma S, Mehrotra A and Murray HW. Circulating Thelper1 (Th1) cell and Th2 cell-associated cytokines in Indian patients with visceral leishmaniasis. Am J Trop Med Hyg. 1997;56:522-25.*
20. *Nylen S, Maurya R, Eidsmo L, Manandhar KD, Sundar S and Sacks D. Splenic accumulation of IL-10mRNA in Tcells distinct from CD4+CD25+ (Foxp3) regulatory T cells in human visceral leishmaniasis. JExpMed. 2007;204:805-17.*
21. *Ansari NA, Saluja S and Salotra P. Elevated levels of interferon-gamma, interleukin-10, and interleukin-6 during active disease in Indian kalaazar. ClinImmunol. 2006;119:339-345.*
22. *Kurkjian KM, Mahmutovic AJ, Kellar KL, Haque R, Bern C and Secor WE. Multiplex analysis of circulating cytokines in the sera of patients with different clinical forms of visceral leishmaniasis. CytometryA.2006;69, 353-358.*
23. *Nylen S and Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis.Trends Immunol. 2007;28:378-84.*
24. *Ansari NA, Kumar R, Gautam S, Nylen S, Singh OP, Sundar S and Sacks D. IL-27 and IL-21 are associated with Tcell IL-10 responses in human visceral leishmaniasis. J Immunol. 2011;186:3977-85.*
25. *Ghalib HW, Whittle JA, Kubin M, Hashim FA, El-Hassan AM, Grabstein KH, Trinchieri G, and Reed SG. IL- 12 enhancesTh1-type responses in human Leishmaniadonovaniinfections.JImmunol.1995;154:4623-29.*
26. *Mansueto P, Vitale G, Di Lorenzo G, Rini GB, Mansueto S, Cillari E. Immunopathology of leishmaniasis: an update. Int J ImmunopatholPharmacol.2007;20:435-45.*
27. *Nascimento MS, Carregaro V, Lima-Junior DS, Costa DL, Ryffel B, Duthie MS, et al. Interleukin 17A acts synergistically with interferon gamma to pro-mote protection against Leishmaniainfantuminfection. J Infect Dis.2015;211:1015-26.*
28. *Gonzalez-Lombana C, Gimblet C, Bacellar O, Oliveira WW, Passos S, Carvalho LP, et al. IL-17 mediates immunopathology in the absence of IL-10 following Leishmaniamajor infection. PLoSPathog. 2013;9:e1003243.*
29. *Ghosh K, Sharma G, Saha A, Kar S, Das PK, Ukil A. Successful therapy of visceral leishmaniasis with curdlan involves T-helper 17 cytokines. J Infect Dis. 2013;207:1016-25.*
30. *Veress B, Omer A, Satir AA, El HassanAM. Morphology of the spleen and lymph nodes in fatal visceral leishmaniasis. Immunology 1977;33:605-10.*
31. *Stern JJ,Oca MJ, Rubin BY, Anderson SL, Murray HW.RoleofL3T4+ and LyT-2+ cells in experimental visceral leishmaniasis.J Immunol. 1988;140:3971-77.*
32. *Tsagozis P, Karagouni E, Dotsika E. CD8(+) T cells with parasite-specific cytotoxic activityandaTc1profileofcytokine and chemokine secretion developing experimental visceral leishmaniasis. ParasiteImmunol. 2003;25:569-79.*
33. *Tsagozis P, Karagouni E, Dotsika E. Function of CD8+ T lymphocytes in a self-curing mouse model of visceral leishmaniasis. Parasitol Int.2005;54:139-46.*
34. *Polley R, Stager S, Prickett S, Maroof A, Zubairi S, Smith DF, Kaye PM. Adoptive immunotherapy against experimental visceral leishmaniasis with CD8+ T cells requires the presence of cognate antigen. Infect Immun.2006;74:773-6.*
35. *Stager S, Smith DF, Kaye PM. Immunization with a recombinant stage regulated surface protein from Leishmania donovani induces protection against visceral leishmaniasis. J Immunol. 2000;165:7064-71.*
36. *Campanelli AP, Roselino AM, Cavassani KA, Pereira MS, Mortara RA, Brodskyn CI, et al. CD4+CD25+ T cells in skin lesions of patients with cutaneous leishmaniasis exhibit phenotypic and functional characteristics of natural regulatory T cells. J Infect Dis. 2006;193:1313-22.*
37. *Anderson CF, Mendez S, Sacks DL. Nonhealing infection despite Th1 polarization produced by a strain of Leishmania major in C57BL/6 mice.J Immunol. 2005;174:2934-41.*
38. *Srivastava S, Pandey SP, Jha MK, Chandel HS, Saha B. Leishmaniaexpressed lipophosphoglycan interacts with toll-like receptor (TLR)-2 to decrease TLR-9 expression and reduce anti-leishmanial responses. ClinExpImmunol. 2013;172:403-9.*

September 2018;5(9) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1437512 Impact Factor: 4.054

39. *Weinkopff T, Mariotto A, Simon G, Hauyon-La Torre Y, Auderset F, Schuster S, et al. Role of Toll-like receptor 9 signaling in experimental Leishmaniabraziliensisinfection. Infect Immun. 2013;81:1575-84.*
40. *Raman VS, Bhatia A, Picone A, Whittle J, Bailor HR, O'Donnell J, et al. Applying TLR synergy in immunotherapy: implications in cutaneous leishmaniasis. J Immunol. 2010;185:1701–10.*
41. *Kumar R, Engwerda C. Vaccines to prevent leishmaniasis. ClinTranslImmunol. 2014;3:e13.*
42. *Das S, Pandey K, Kumar A, Sardar AH, Purkait B, Kumar M, Kumar S, Ravidas VN, Roy S, Singh D, Das P.TGF-β1 re-programs TLR4 signaling in L. donovani infection: enhancement of SHP-1 and ubiquitin-editing enzyme A20. Immunol Cell Biol. 2012;90:640-54.*
43. *SavoiaD.Recent updates and perspectives on leishmaniasis.J Infect Dev Ctries. 2015;9:588–96.*
44. *Sundar S, Singh A, Singh OP. Strategies to overcome antileishmanial drugs unresponsiveness. Re Dai Yi XueZaZhi. 2014;2014:646932.*
45. *Engwerda CR, Matlashewski G. Development of Leishmaniavaccines in the era of visceral leishmaniasiselimination.Trans R Soc Trop Med Hyg. 2015;109:423-4.*
46. *Chappuis F, Sundar S, Hailu A, et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol. 2007;5:873-82.*
47. *Mohan T, Verma P, Rao DN. Novel adjuvants & delivery vehicles for vaccines development: a road ahead. Indian J Med Res. 2013;138:779-795.*
48. *Abdian N, Gholami E, Zahedifard F, Safaee N, Rafati S. Evaluation of DNA/DNA and prime-boost vaccination using LPG3 against Leishmania major infection in susceptible BALB/c mice and its antigenic properties in human leishmaniasis. Exp. Parasitol. 2011;127:627.*
49. *Araújo MS, de Andrade RA, Sathler-AvelarR,Magalhães CP, Carvalho AT, Andrade MC, Campolina SS, Mello MN, Vianna LR, Mayrink W, ReisAB, Malaquias LC, Rocha LM, Martins-Filho OA. Immunological changes in canine peripheral blood leukocytes triggered by immunization with first or second generation vaccines against canine visceral leishmaniasis. Vet. Immunol.Immunopathol. 2011;141:64.*
50. *Nagill R, Kaur S. Vaccine candidates for leishmaniasis: a review. Int. Immunopharmacol. 2011;11: 1464.*
51. *Singh B and Sundar S. Leishmaniasis: vaccine candidates and perspectives.Vaccine. 2012.30, 3834.*
52. *Singh OP, Stober CB, Singh AK, Blackwell JM, Sundar S. Cytokineresponses to novel antigens in an Indian population living in an areaendemic for visceral leishmaniasis. PLoSNegl Trop Dis. 2012;6:e1874.*
53. *Mayrink W, Genaro O, Silva JC, da Costa RT, Tafuri WL, Toledo VP, et al. Phase I and II open clinical trials of a vaccine against Leishmaniachagasiinfections in dogs. Mem Inst Oswaldo Cruz.1996;91:695-7.*
54. *Foroughi-Parvar F, Hatam G. Vaccines for canine leishmaniasis. AdvPrev Med. 2014;2014:569193.*
55. *Giunchetti RC, Correa-Oliveira R, Martins-Filho OA, Teixeira-Carvalho A, Roatt BM, de Oliveira Aguiar-Soares RD, et al. Immunogenicity of a killed Leishmaniavaccine with saponin adjuvant in dogs. Vaccine.2007;25:7674-86.*
56. *Giunchetti RC, Correa-Oliveira R, Martins-Filho OA, Teixeira-Carvalho A, Roatt BM, de Oliveira Aguiar-Soares RD, et al. A killed Leishmaniavaccine with sand fly saliva extract and saponin adjuvant displays immunogenicity in dogs. Vaccine2008;26:623-38.*
57. *Bruhn KW, Birnbaum R, Haskell J, Vanchinathan V, Greger S, Narayan R, et al. Killed but metabolically active Leishmaniainfantumas a novel whole-cell vaccine for visceral leishmaniasis. Clin Vaccine Immunol.2012;19:490-8.*
58. *Dey R, Dagur PK, Selvapandiyan A, McCoy JP, Salotra P, Duncan R, et al. Live attenuated Leishmania donovani p27 gene knockout parasites are nonpathogenic and elicit long-term protective immunity in BALB/c mice. J Immunol.2013;190:2138-49.*
59. *Bhattacharya P, Dey R, Dagur PK, Kruhlak M, Ismail N, Debrabant A, et al. Genetically modified live attenuated Leishmania donovani parasites induce innate immunity through classical activation of macrophages that direct the Th1 response in mice. Infect Immun. 2015;83:3800-15.*
60. *Anand S, Madhubala R. Genetically engineered ascorbic acid-deficient live mutants of Leishmania donovani induce long lasting protective immunity against visceral leishmaniasis. Sci Rep.2015;5:10706.*
61. *Fiuza JA, Gannavaram S, Santiago Hda C, Selvapandiyan A, Souza DM, Passos LS, et al. Vaccination using live attenuated Leishmaniadonovanicentrin deleted parasites induces protection in dogs against Leishmaniainfantum. Vaccine.2015;33:280-8.*

September 2018;5(9) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1437512 Impact Factor: 4.054

62. *Seyed N, Zahedifard F, Safaiyan S, Gholami E, Doustdari F, Azadmanesh K, et al. In silico analysis of six known Leishmaniamajor antigens and in vitro evaluation of specific epitopes eliciting HLA-A2 restricted CD8 T cell response.PLoSNegl Trop Dis. 2011;5:e1295.*
63. *Mazumder S, Maji M, Ali N. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: high immunogenicity and protection. PLoSNegl Trop Dis.2011;5:e1429.*
64. *Elfaki ME, Khalil EA, De Groot AS, Musa AM, Gutierrez A, Younis BM, et al. Immunogenicity and immune modulatory effects of in silico predicted L. donovani candidate peptide vaccines. Hum VaccinImmunother.2012; 8:1769-74.*
65. *Ravindran R, Maji M, Ali N. Vaccination with liposomal leishmanial antigens adjuvanted with monophosphoryl lipid-trehalosedicorynomycolate (MPL- TDM) confers long-term protection against visceral leishmaniasis through a human administrable route. Mol Pharm. 2012;9:59-70.*
66. *Gupta R, Kushawaha PK, Tripathi CD, Sundar S, Dube A. A novel recombinantLeishmaniadonovanip45, a partial coding region of methionine aminopeptidase, generates protective immunity by inducing a Th1 stimulatory response against experimental visceral leishmaniasis. Int J Parasitol. 2012;42:429-35.*
67. *Choudhury R, Das P, De T, Chakraborti T. 115 kDa serine protease confers sustained protection to visceral leishmaniasis caused by Leishmania donovani via IFN-gamma induced down-regulation of TNF-alpha mediated MMP-9 activity. Immunobiology.2013;218:114-26.*
68. *Resende LA, Roatt BM, Aguiar-Soares RD, Viana KF, Mendonca LZ, Lanna MF, et al. Cytokine and nitric oxide patterns in dogs immunized with LBSap vaccine, before and after experimental challenge with Leishmaniachagasiplus saliva of Lutzomyialongipalpis. Vet Parasitol. 2013;198:371-81.*
69. *Ramirez L, Santos DM, Souza AP, Coelho EA, Barral A, Alonso C, et al. Evaluation of immune responses and analysis of the effect of vaccination of the Leishmaniamajor recombinant ribosomal proteins L3 or L5 in two different murine models of cutaneous leishmaniasis. Vaccine. 2013;31:1312-9.*
70. *Fernandes CB, Junior JT, de Jesus C, Souza BM, Larangeira DF, Fraga DB, et al. Comparison of two commercial vaccines against visceral leishmaniasis in dogs from endemic areas: IgG, and subclasses, parasitism, and parasite transmission by xenodiagnosis. Vaccine. 2014;32:1287-95.*
71. *Testasicca MC, dos Santos MS, Machado LM, Serufo AV, Doro D, Avelar D, et al. Antibody responses induced by Leish-Tec(R), an A2-based vaccine for visceral leishmaniasis, in a heterogeneous canine population. Vet Parasitol.2014; 204:169-76.*
72. *Das A, Ali N. Combining cationic liposomal delivery with MPL-TDM for cysteine protease cocktail vaccination against Leishmania donovani: evidence for antigen synergy and protection. PLoSNegl Trop Dis.2014;8:e3091.*
73. *Katebi A, Gholami E, Taheri T, Zahedifard F, Habibzadeh S, Taslimi Y, et al. Leishmaniatarentolaesecreting the sand fly salivary antigen PpSP15 confers protection against Leishmania major infection in a susceptible BALB/c mice model. MolImmunol. 2015 67(2 Pt B):501-11.*
74. *Pereira L, Abbehusen M, Teixeira C, Cunha J, Nascimento IP, Fukutani K, et al. Vaccination with Leishmaniainfantumacidic ribosomal P0 but not with nucleosomal histones proteins controls Leishmaniainfantuminfection in hamsters. PLoSNegl Trop Dis. 2015;9:e0003490.*
75. *Miura R, Kooriyama T, Yoneda M, Takenaka A, Doki M, Goto Y, et al. Efficacy of recombinant canine distemper virus expressing Leishmania antigen against Leishmania challenge in dogs. PLoSNegl Trop Dis.2015;9:e0003914.*
76. *Athanasiou E, Agallou M, Tastsoglou S, Kammona O, Hatzigeorgiou A, Kiparissides C, et al. A poly(lacticcoglycolic) acid nanovaccine based on chimeric peptides from different Leishmaniainfantumproteins induces dendritic cells maturation and promotes peptide-specific IFNγ-producing CD8+ T cells essential for the protection against experimental visceral leishmaniasis. Front Immunol.2017;8:684.*
77. *Mazumder S, Maji M, Das A, Ali N. Potency, efficacy and durability of DNA/ DNA, DNA/protein and protein/protein based vaccination using gp63 against Leishmania donovani in BALB/c mice. PLoS One.2011;6:e14644.*

September 2018;5(9) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1437512 Impact Factor: 4.054

78. *Doroud D, Zahedifard F, Vatanara A, Najafabadi AR, Taslimi Y, Vahabpour R, et al. Delivery of a cocktail DNA vaccine encoding cysteine proteinases typeI, II and III with solid lipid nanoparticles potentiate protective immunity against Leishmania major infection. J Control Release. 2011;153:154-62.*
79. *Hugentobler F, Di Roberto RB, Gillard J, Cousineau B. Oral immunization using live Lactococcuslactiscoexpressing LACK and IL-12 protects BALB/c mice against Leishmania major infection. Vaccine. 2012;30:5726-32.*
80. *Kushawaha PK, Gupta R, Tripathi CD, Sundar S, Dube A. Evaluation of Leishmaniadonovaniprotein disulfide isomerase as a potential immunogenic protein/vaccine candidate against Visceral Leishmaniasis. PLoS One. 2012;7:e35670.*
81. *Saljoughian N, Taheri T, Zahedifard F, Taslimi Y, Doustdari F, Bolhassani A, et al. Development of novel prime-boost strategies based on a tri-gene fusion recombinant L. tarentolaevaccine against experimental murine visceral leishmaniasis. PLoSNegl Trop Dis. 2013;7:e2174.*
82. *Shahbazi M, Zahedifard F, Saljoughian N, Doroud D, Jamshidi S, Mahdavi N, et al. Immunological comparison of DNA vaccination using two delivery systems against canine leishmaniasis. Vet Parasitol. 2015;212:130-9.*
83. *Shahbazi M, Zahedifard F, Taheri T, Taslimi Y, Jamshidi S, Shirian S, et al. Evaluation of live recombinant nonpathogenic Leishmaniatarentolaeexpressing cysteine proteinase and A2 genes as a candidate vaccine against experimental canine visceral leishmaniasis. PLoS One. 2015;10:e0132794.*
84. *Tabatabaie F, Mahdavi M, Faezi S, Dalimi A, Sharifi Z, Akhlaghi L, et al. Th1 Platform immune responses against Leishmania major induced by thiol specific antioxidant based DNA vaccines. Jundishapur J Microbiol. 2014;7:e8974.*
85. *Campos BL, Silva TN, Ribeiro SP, Carvalho KI, Kallas EG, Laurenti MD, et al. Analysis of iron superoxide dismutase-encoding DNA vaccine on the evolution of the Leishmaniaamazonensisexperimental infection. Parasite Immunol.2015;37:407-16.*
86. *Riede O, Seifert K, Oswald D, Endmann A, Hock C, Winkler A, et al. Preclinical safety and tolerability of a repeatedly administered human leishmaniasis DNA vaccine. Gene Ther. 2015; 22:628-35.*
87. *Mukhopadhyay S, Sen P, Bhattacharyya S, Majumdar S, Roy S. Immunoprophylaxis and immunotherapy against experimental visceral leishmaniasis. Vaccine. 1999;17:291-300.*
88. *Mukhopadhyay S, Bhattacharyya S, Majhi R, et al. Use of an attenuated leishmanial parasite as an immunoprophylactic and immunotherapeutic agent against murine visceral leishmaniasis. ClinDiagn Lab Immunol. 2000;7:233-240.*
89. *Musa AM, Khalil EA, Mahgoub FA, et al. Immunochemotherapy of persistent post-kala-azar dermal leishmaniasis: a novel approach to treatment. Trans R Soc Trop Med Hyg. 2008;102:58-63.*
90. *Datta S, Manna M, Khanra S, et al. Therapeutic immunization with radio-attenuated Leishmaniaparasites through i.m. route revealed protection against the experimental murine visceral leishmaniasis. Parasitol Res. 2012;111:361-9.*
91. *Datta S, Roy S, Manna M. Therapy with radio-attenuated vaccine in experimental murine visceral leishmaniasis showed enhanced T cell and inducible nitric oxide synthase levels, suppressed tumor growth factor beta production with higher expression of some signaling molecules. Braz J Infect Dis. 2015;19:36-42.*
92. *Santos WR, Aguiar IA, Paraguai de Souza E, de Lima VM, Palatnik M, Palatnik-de-Sousa CB.Immunotherapy against murine experimental visceral leishmaniasis with the FML-vaccine.Vaccine. 2003;21:4668–76.*
93. *Borja-Cabrera GP, Cruz Mendes A, Paraguai de Souza E, et al. Effective immunotherapy against canine visceral leishmaniasis with the FMLvaccine. Vaccine.2004;22:2234-43.*
94. *Santos FN, Borja-Cabrera GP, Miyashiro LM, et al. Immunotherapy against experimental canine visceral leishmaniasis with the saponin enriched-Leishmunevaccine.Vaccine. 2007;25:6176-90.*
95. *Neogy AB, Vouldoukis I, da Costa JM, Monjour L. Exploitation of parasite-derived antigen in therapeutic success against canine visceral leishmaniosis.Veterinary Group of Lupino.Vet Parasitol. 1994;54:367-73.*
96. *Gamboa-Leon R, Paraguai de Souza E, Borja-Cabrera GP, et al. Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase-DNA vaccine of Leishmaniadonovani. Vaccine. 2006;24:4863–73.*

September 2018;5(9) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.1437512 Impact Factor: 4.054

97. *Miret J, Nascimento E, Sampaio W, et al. Evaluation of an immune chemotherapeutic protocol constituted of N-methyl meglumineantimoniate (Glucantime) and the recombinant Leish-110f + MPL-SE vaccine to treat canine visceral leishmaniasis. Vaccine.2008;26:1585-94.*
98. *Bhaumik SK, Naskar K, De T. Complete protection against experimental visceral leishmaniasis with complete soluble antigen from attenuated Leishmaniadonovanipromastigotes involves Th1-immunity and downregulation of IL-10. Eur J Immunol. 2009;39:2146-60.*
99. *Trigo J, Abbehusen M, Netto EM, et al. Treatment of canine visceral leishmaniasis by the vaccine Leish-111f+MPL-SE. Vaccine. 2010;28:3333-40.*
100. *Borja-Cabrera GP, Santos FN, Santos FB, et al. Immunotherapy with the saponin enriched-Leishmune vaccine versus immunochemotherapy in dogs with natural canine visceral leishmaniasis.Vaccine. 2010;28:597-603.*
101. *Ferreira JH, Silva Ldos S, Longo-Maugeri IM, Katz S, Barbieri CL. Use of a recombinant cysteine proteinase from Leishmania (Leishmania) infantumchagasifor the immunotherapy of canine visceral leishmaniasis. PLoSNegl Trop Dis. 2014;8:e2729.*
102. *Joshi J, Kaur S. To investigate the therapeutic potential of immunochemotherapy with cisplatin + 78 kDa + MPL-A against Leishmaniadonovaniin BALB/c mice.Parasite Immunol. 2014;36:3-12.*
103. *Seifert K, Juhls C, Salguero FJ, Croft SL. Sequential chemoimmunotherapy of experimental visceral leishmaniasis using a single low dose of liposomal amphotericin B and a novel DNA vaccine candidate. Antimicrob Agents Chemother. 2015;59:5819-23.*
104. *Didwania N, Shadab M, Sabur A, Ali N.Alternative to Chemotherapy-The Unmet Demand againstLeishmaniasis.Front Immunol. 2017;8:1779.*
105. *Beaumier CM, Gillespie PM, Hotez PJ, Bottazzi ME. New vaccines for neglected parasitic diseases and dengue. Transl Res. 2013;162:144-55.*
106. *Llanos-Cuentas A, Calderón W, Cruz M, Ashman JA, Alves FP, Coler RN et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine when used in combination with sodium stibogluconate for the treatment of mucosal leishmaniasis. Vaccine. 2010;28:7427-35.*
107. *Nascimento E, Fernandes DF, Vieira EP, Campos-Neto A, Ashman JA, Alves FP,et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1 + MPL-SE vaccine when used in combination with meglumineantimoniatefor the treatment of cutaneous leishmaniasis. Vaccine. 2010;28:6581-7.*
108. *Jain K, Jain NK. Vaccines for visceral leishmaniasis: a review. J Immunol Methods. 2015.*
109. *MuLeVaClin. Multivalent vaccine for human visceral leishmaniasis; 2015.*

Prophylactic Vaccine against VL

Table 1: Prophylactic vaccine used against Leishmaniasis

Antigen	Vaccine approaches	Animal model	Adjuvant	refer
1st generation anti-leishmanial vaccine				
Merthiolated sound-disrupted *Leishmaniabraziliensis*	Killed vaccine	Dogs	BCG	[53, 54]
Killed *L. braziliensis*	Killed vaccine	Dogs	Saponin	[55, 56]
KBMA *Leishmaniainfantumchagasi*	Killed vaccine	Mouse	-	[57]
p27 gene knockout *L. donovani* parasites	Live attenuated	mouse	-	[58]
Live mutants of *Leishmania* lacking genes like dihydrofolate reductase, biopterin reductase, and cysteine proteases (CPs)	Live attenuated	mouse	-	[59]
Ascorbic acid-deleted live mutants of *L. donovani*	Live attenuated	Mouse	-	[60]
Centrin-deficient parasites of *L. donovani*	Live attenuated	Dogs	-	[61]

Indian Journal of Medical Research and Pharmaceutical Sciences
September 2018;5(9) ISSN: ISSN: 2349-5340
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2nd generation anti-leishmanial vaccine				
Chimeric peptides containing HLA-A2 restricted epitopes from six immunogenic L. major proteins(CPB, CPC, LmsTI1, TSA, LeIF and LPG-3)	Peptide Vaccine			[62]
*L. donovani*surface GP63	Recombinant protein vaccine	Mouse, Human	MPL-TDM, CGP-ODN	[63, 64]
Soluble leishmanial antigens of *L. donovani* promastigotes	Fractionated vaccine	Mouse	MPL-TDM	[65]
*L. donovani*p45 (rLdp45)	Recombinant protein	Hamster and Human	-	[66]
115 kDa soluble serine protease	Fractionated vaccine	Mouse	IL-12	[67]
*L. braziliensis*promastigote proteins	Fractionated vaccine	Dog	Saponin	[68]
Leishmania major ribosomal protein L3 or L5	Recombinant protein	Mouse	CpG-ODN	[69]
Leishmune (purified *L. donovani* fraction FML)	Fractionated vaccine	Dog	Saponin	[70]
Leish-Tec (*L. donovani* amastigote-specific protein A2)	Recombinant protein	Dog	Saponin	[71]
Cocktail of *L. donovani* CPs types I, II, and III	Recombinant protein cocktail vaccine	Hamster	MPL-TDM	[72]
Recombinant *L. tarentolae* secreting PpSP15	Recombinant vaccine	Mouse	CPG-ODN	[73]
*L. infantum*acidic ribosomal P0	Recombinant protein	Hamster	-	[74]
Cocktail of rCDV-LACK, rCDV-TSA, and rCDV-LmSTI1	Recombinant protein cocktail vaccine	Dog	-	[75]
Chimeric peptides containing HLA-restricted epitopes from three immunogenic *L. infantum* proteins (CPA, histone H1 and KMP11)	Peptide vaccine	Transgenic mice	Poly (lactic-co-glycolic acid nanoparticles and/or MPL-A	[76]
3rd generation anti-leishmanial vaccine				
*L. donovani*surface GP63	DNA vaccine and T-cell epitope DNA vaccine	Mouse, Human	MPL-TDM, CGP-ODN	[63, 77]
Cocktail of *L. major* CPs type I, II, and III	Cocktail DNA vaccine	Mouse	MPL-TDM	[78]
*Lactobacillus lactis*expressing LACK and mouse IL-12	Recombinant vaccine	Mouse		[79]
Recombinant *L. donovani* protein disulfide isomerase	DNA vaccine	Hamster and Human	-	[80]

Indian Journal of Medical Research and Pharmaceutical Sciences
September 2018;5(9) ISSN: ISSN: 2349-5340
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*Leishmaniatarentolae*expressing *L. donovani*A2 antigen along with CPs [CPA and CPB without its unusual C-terminal extension (CPB-CTE)]	Recombinant vaccine and DNA vaccine	Mouse, Dogs	-	[81, 82, 83]
L. major TSA	DNA vaccine	mouse	Aluminium phosphate	[84]
*Leishmaniaamazonensis*iron superoxide dismutase	DNA vaccine	mouse	-	[85]
T-cell epitope of KMP11, CPA, CPB, EF1α, and TSA (LEISHDNAVAX)	Multiantigenic T-cell epitope fusion DNA vaccine	Mouse	-	[86]

Table 2

Antigen	**Vaccine approaches**	**Animal model**	**Adjuvent**	**References**
1st generation				
L. donovani/ amastigote UR6	Live/sonicated parasite	Hamster	-	[87]
L. donovani/ amastigote UR6	Live/sonicated parasite	BALB/c mice	-	[88]
Alum/Autoclaved *L. major*	killed parasite	Human PKDL patients (field trial in Sudan)	Bovine Calmette-Guerin	[89]
L. donovani promastigote	Attenuated parasite	BALB/c mice	-	[90, 91]
2nd generation				
Fucose mannose ligand (FML)	glycoprotein	BALB/c mice	saponin	[92]
FML	glycoprotein	Mongrel dogs (experimental; Brazil)	saponin	[93]
Leishmune® (FML)	glycoprotein	Mongrel dogs (experimental; Brazil)	saponin	[94]
L. infantum-derived Fraction-2	Fractionated protein vaccine	Naturally infected dogs (France)	-	[95]
NH-DNA	glycoprotein	BALB/c mice	Aqueous garlic extract	[96]
Leish-110f	Polyprotein vaccine	Naturally infected mongrel dogs (Brazil)	Monophosphoryl Lipid A plus squalene (MPL-SE)	[97]
Complete Soluble Antigen (CSA)	Protein vaccine	BALB/c mice	-	[98]
Soluble Leishmania Antigen	Protein vaccine	BALB/c mice	Pulsed Dendritic	[98]

Indian Journal of Medical Research and Pharmaceutical Sciences
September 2018;5(9) ISSN: ISSN: 2349-5340
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(SLA)			Cell	
Leish-111f	Polyprotein vaccine	Naturally infected dogs (Brazil)	MPL-SE	[99]
Leishmune	Protein vaccine	Naturally infected dogs (Brazil)	-	[100]
recombinant cysteine proteinase from *L. (L.) infantumChagasi*rLdccys1+ *P. acnes*	recombinant protein vaccine	Naturally infected dogs (Brazil)	-	[101]
GRP78	Protein vaccine	BALB/c mice	Monophosphoryl Lipid A (MPLA)	[102]
3rd generation				
LEISHDNAVAX	DNA vaccine	C57BL/6J mice		[102]