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Identifying vaccine targets for anti-leishmanial vaccine development

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Abstract

Leishmaniasis is a neglected tropical disease spread by an arthropod vector. It remains a significant health problem with an incidence of 0.2–0.4 million VL and 0.7–1.2 million CL cases each year. There are limitations associated with the current therapeutic regimens for leishmaniasis and the fact that after recovery from infection the host becomes immune to subsequent infection therefore, these factors forces the feasibility of a vaccine for leishmaniasis. Publication of the genome sequence of *Leishmania* has paved a new way to understand the pathogenesis and host immunological status therefore providing a deep insight in the field of vaccine research. This review is an effort to study the antigenic targets in *Leishmania* to develop anti-leishmanial vaccine.

Keywords

Leishmania; arthropod; vaccine; pathogenesis

1. Introduction

Leishmaniases area group of protozoan disease, transmitted by the bite of female phlebotomine sand fly. It is represented by a broad spectrum of clinical manifestations ranging from ulcerative skin lesions developing at the site of sand fly bite (localized cutaneous leishmaniasis), multiple non-ulcerative nodules (diffuse cutaneous leishmaniasis), destructive mucosal inflammation (mucosal leishmaniasis), to disseminated visceral disease (visceral leishmaniasis, VL). VL is a systemic infection characterized by prolonged fever, cachexia, hepatosplenomegaly, hypergammaglobulinemia, and pancytopenia [1]. If left untreated, all clinically symptomatic patients die within months; even after initial cure from VL, some of individuals in Indian subcontinent and Sudan may develop cutaneous manifestations in the form of skin lesions containing the parasites, termed as post-kala-azar dermal leishmaniasis (PKDL). Of the 200–400 thousand new cases of VL reported every year, 90% occur in Bangladesh, Brazil, Ethiopia, India and Sudan [2] ; India alone contributes for more than half of the annual global VL cases.

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1.1.Need for a vaccine

Leishmaniasis has emerged as a major public health problem throughout the world because of several therapeutic challenges such as high cost, drug toxicity and growing parasite resistance to currently available drugs.

Vaccination should be the driving force for controlling leishmaniasis because the recovery from infection confers immunity to reinfection [3], but still no human *Leishmania* vaccine is available today [4] because major hurdle is the lack of experimental animal models that tightly mimic the disease as it occurs in humans [5]. The major challenges confronting the scientific community for VL vaccine development includes- host evasive mechanisms employed by the parasite and heterogeneity in human population. Absence of an *in vivo* animal model accurately reflects the clinical features of human VL [6] adding to the limitations of development of immunoprophylaxis against VL. However, current advances in genome sequencing and biotechnology have provided renewed thrust for VL vaccinology, an area which has been neglected for years. Development of a cross-protective anti-*Leishmania* vaccine should be the public health goal to combat this lethal infection.

1.2.Immunology of VL and progress towards vaccinology

The advances in understanding the cell-mediated immune responses have made the study of vaccine candidates easier. The immune response raised against *Leishmania* is dependent on the species of the parasite infecting the host and the genetics of the host [7]. There are several factors influencing the immune response to infection but TH1-cells are the major players deciding the fate of infection [8] as there is tug-of-war like situation between the host's ability to mount TH1 responses and the ability of parasite to evade and manipulate it [9]. Other cells that are considered for their vital roles in immune response to *Leishmania* infection includes macrophages, dendritic cells (DC) [10], natural killer (NK) cells [11], T-helper cells, cytotoxic T cells, and the cytokines secreted by these cells; CD8 cells are known to control the infection by secreting IFN-γ that activates macrophages and also promotes the differenciation of naive T-cells into TH1 [12] that directly contributes in controlling the infection hence driving towards the development of protective immune responses.

Macrophages, serving as hosts for *Leishmania*, are activated by IFN-γ [13] from TH1 and NK cells, for killing the parasite on phagocytosis. Opsonisation is known to kill the parasite but in the context of leishmanial infection C3 component of complement (meant for opsonisation) fails to do so and this process renders the parasite survival in the non-activated macrophages [14]. DC present the antigens to CD4+ T cells (T-helper cells) and CD8+ T cells (cytotoxic T cells) [15], and decides the fate of leishmanial infection by driving the differentiation and proliferation of T-helper cells to either TH1 or TH2 ; the presentation of antigen to CD4+ cells leads to secretion of IL-12 that drives the proliferation of (IFN-γ secreting) TH1 cells and NK cells thus, activating the macrophages and inhibiting the TH2 responses [16]. On the other hand, there are growing evidences indicating the role of IL-10 and TGF- beta in down modulating type 1 responses, resulting in the persistence of parasite in the host and intensifying the disease manifestation [17].

The progress towards vaccine includes an era from leishmanization to subunit protein and DNA based vaccines. Leishmanization, the age old practice of inoculating virulent parasites in the individuals was the major landmark in the vaccine development programme followed by the use of whole parasite killed vaccine but certain limitations fuelled the research for the second generation vaccine using defined parasite fractions or protein was started with varying range of protective immune responses (driving the production of IFN-γ and preventing the TH2- skewed responses which causes IL-10 production that promotes the infection) [18] ; the most important of which has entered the clinical trial is Leish-111f, other candidate vaccine is Leishmune, with the credit of being the first prophylactic vaccine in the field. The other hotspots in this regard includes the DNA based vaccine and DC-pulsed with sandfly saliva or parasite protein.

1.3.Immune Evasion by Leishmania

Survival of *Leishmania* within the mammalian host depends on its ability of evasion of immunity barriers. Being intracellular parasite, *Leishmania* directly evades the humoral immune responses but become susceptible to attack by the cell mediated immunity [19]. As a part of its subversive activity, *Leishmania* inhibits the production of interleukin-12 (IL-12) which is necessary for the activation of macrophages and subsequent leishmanicidal activity by up-regulating the inducible nitric oxide synthase (iNOS), nitric oxide (NO) and IFN- γ [20].

On the other hand IFN-γ producing TH1-cells are amongst the major weapons to deploy against the parasite, but on infection the parasite directly interferes with the functioning of these cells thereby overcoming the host immune mechanisms. The cell-mediated immunity employs CD-8 cells that are known to eliminate the parasite but death and exhaustion of parasite specific CD8-cells has been reported in VL. [21]. As a part of evasion mechanism parasite is also known to regulate cytokine responses in its favour (upregulating IL-10 and TGF-β) and reducing the production of IL-1 and TNF-α along with its effect on antigen processing and presentation. Various weapons, employed by the invading parasite, demonstrate for the evolution of the parasite to successfully evade the immune responses designed to target and destroy them [22]. Such evasion mechanisms include all the strategies from the modulation of macrophage function to the inhibition of antigen presentation and T cell stimulation. Therefore, improved understanding of these parasite adaptation mechanisms will assist in the development of an effective prophylactic vaccine, with the potential of inducing the specific immune responses required to eliminate the parasite.

2. Development of Vaccine

Development of a vaccine includes five distinct stages: discovery, pre-clinical development, clinical-development, registration, and postmarketing evaluation of the prepared vaccine. The whole five steps protocol may be limited by many factors, amongst them the major forces are-

- Cost of development- The high cost of developing a vaccine has been the major limiting factor that has hampered the vaccine development programme although many organisations are funding to promote the research in this field.

- Political involvement- NTD's are basically localised to the developing countries affecting the poor communities thus, private companies do not find much profit which calls for the involvement of the public resources for the development in this field.

The important features that an ideal antileishmanial vaccine should possess include- safety, low cost, ease of production, capability of inducing robust, long-term T cell responses, and both prophylactic and therapeutic efficacy. Ideally, such vaccine would offer cross-species effectiveness against CL and VL. As this might not be feasible, the development of a VL-specific vaccine remains an important global health priority. In this context significant efforts have been made by the scientific communities including the development and trials of first and second generation vaccines.

2.1. First-Generation Vaccines

The first vaccine studies in the field of leishmaniasis started in 1940's which included an ancient practise of administration of virulent *Leishmania* parasites to discrete skin location of an uninfected individual, ensuing to self-healing cutaneous lesion known as leishmanization. It produced successful results in initial trials in Soviet Union, Asia, and the Middle East, with 100% efficacy. However, it was discontinued due to safety issues associated with the use of live vaccines, as well as its immunosuppressant activity in children towards the vaccines for tetanus, diphtheria and pertussis [23], and development of large uncontrolled skin lesions.

Live virulent form of vaccine (leishmanization) was later replaced by first-generation *Leishmania* vaccine, composed of killed parasites or live attenuated *Leishmania* parasites. Killed parasites as vaccine was first demonstrated by Brazilian scientists and later by Mayrink and colleagues developed a killed vaccine with good safety and immunogenicity but it conferred small degree of protection [24]. Convit et al. in early 1990s used the combined therapeutic approach using the killed *L. amazonensis or L. mexicana* and Bacillus Calmette Guerin (BCG) for the treatment of cutaneous leishmaniasis (CL) with significantly high cure rate (>95%) [25]. These killed vaccines are simple and relatively easy to produce in developing countries at low cost. This was one of the advantages that made them attractive candidates for vaccine development. However, the difficulty of standardization of vaccines derived from cultured parasites is a potential obstacle in the way of their registration. There remains a suspicion about the reproducibility of their protective efficacy.

In a series of randomised trials in new (Ecuador, Columbia, Brazil) and old (Sudan and Iran) worlds using the killed parasite vaccine resulted in the variable efficacy associated with their inability to produce long-lasting immune responses for protection. In contrast to the prophylactic trials of first generation vaccines (as mentioned above) the therapeutic trials have shown very encouraging results [26] [27] therefore, justifying that fact that first generation leishmaniasis vaccines have their utility for therapeutic purposes.

Live attenuated form of the *Leishmania* is another form of vaccine where the parasites are genetically modified to get a less virulent form of *Leishmania* parasites, yet maintain immunogenicity. It is an appealing approach because such parasites closely mimic natural

infection thus producing similar immune responses without the threat associated with live virulent parasites. This form of vaccine is basically produced by replacing or removing of essential genes required by the parasite therefore, termed as knock out parasites [28, 29]. The first innovation in this field was the production of knock-out lacking reductase thymidylate synthase gene (DHFR-TS) in *L. major* with significant protection in mice but it was unsuccessful in primates [30]. There are concerns about these vaccine regarding the reversion of the virulent forms of the parasite, or targeted deletion of virulence genes resulting in complete destruction of the parasite or mutants that only delay lesion development [31]. However, the most recent advancement in this field includes the use of killed but metabolically active (KBMA) form of parasites produced by treatment with amotosalen and low-dose UV radiation that are replication deficient with protective abilities in mice against *L. infantum* thus, serving as a novel whole-cell vaccination approach [32].

2.2. Second and Third -generation vaccines

The second-generation vaccine for *Leishmania* includes broad category ranging from recombinant proteins to polyproteins. These vaccines have been evaluated widely, ranging from genetically modified parasites (either knock out [33] or with suicidal cassettes along with the introduction of drug sensitive genes [34]), recombinant bacteria [35] or virus [36] as delivery agents expressing theparasite antigens. Some other molecular advances suggests for the use of live attenuated vaccines as centrin-knockout, attenuated form of parasite with good efficacy in terms of immunogenicity [37]. There are other forms of second generation vaccines as purified *Leishmania* sub-fractions that may include the proteins or lipophosphoglycan from parasite. All these forms have one or the other limitations that prevents their use as immunoprophylactic and/or immunotherapeutic vaccine but the most recent advancement in this context is the use of recombinant vaccines like Leish-111f, with encouraging results, proved to be safe and immunogenic in healthy volunteers in the US, Brazil, Peru [38] and India [39] (Table 2). The major success in second generation vaccine included Leishmune (FML vaccine) [40] and Leish-Tec (adenovirus with L. donovani A2 protein) [41] which are licensed for the canids in Brazil.

DNA based vaccines constitute the third generation vaccine where the gene encoding for a target protein is cloned in an expression vector. The protein is capable of providing good degree of protection as being highly immunogenic with higher stability in comparison to the second generation vaccines (recombinant proteins). The first DNA vaccine was prepared for gp63 with significant levels of CD4-responses [42]. There are other parasite derived molecules as LACK, PSA-2 used as DNA vaccine enlightening new hope in the horizon of vaccine development (discussed in detail below) the most recent progress in this field illustrates the use of leishmanial haemoglobin-receptor (HbR) as vaccine candidate [43]; these vaccines are available with various modifications –heterologous prime-boost vaccination [44] or addition of oligodeoxynucleotides (ODN) or cytokines [45].

2.2.1. Virulence factors in parasite as vaccine candidates: The significance—

Based on virulence factors of the *Leishmania* parasite, a number of experimental vaccines were tested in the mouse model [46]. The major challenge confronting the scientific community in this field is the differences in the new and the old world parasites despite

similarities in the clinical illness e.g. *L. major* and *L. mexicana/L. amazonensis*, are markedly different; lipophsphoglycan (LPG) is a virulence factor for *L. major*, but not for *L. mexicana* [47] ;there remains the functional difference in the immune response evoked by the parasites from old and new worlds during the infection. TH1 responses has been documented for its protective role, but *L. amazonensis* is an exception to this rule where the parasite persists in the presence of dominant TH1 response, and causes minimal disease in the complete absence of T cells. These findings underscore the major gaps in the differences in the immunobiology of parasites that seemingly cause similar disease.

There are other issues concerning the vaccine development and their comparative evaluation that states for the important characteristics for designing a candidate vaccine-antigen, parasite strain, parasite load of challenge, plasmid backbone (in case of recombinant or DNA vaccine), number of vaccine doses to be administered before challenge, experimental procedure, murine model adopted etc., as these factors determine different outcomes in different studies. In this context a large number of molecules have been studied and identified to date as potential vaccine targets in the direction to develop a vaccine for the leishmaniasis, of which the most promising vaccine candidates are chosen after testing in murine models are included for discussion in this review : (gp63), LACK, parasite surface antigen (PSA), *Leishmania*-derived recombinant polyprotein (LEISH–F3 + GLA-SE, *Leish*-111f and KSAC), serine proteases and other candidate antigens (Table 1). So this review will highlight the potent antigenic molecules with their ability to serve as vaccine targets for the cure of leishmanial infections. As a part of second generation vaccine these molecules may be categorised on the basis of the pathways/origin of antigenic proteins:

2.2.2. Vaccine targets based on Excreted and Secreted (ES) antigens—The parasite releases a vast range of molecules at different life stages in its surrounding environment to manipulate and make it conducive for its survival by inactivating the macrophages and to some extent altering the host cell signalling mechanisms; in this context the major excretory or secretory products are included as the important targets as they are thought to have crucial role in the biology or virulence of *Leishmania* parasites. These molecules can be processed into peptides, and in association with MHCI/MHCII molecules, triggering the host cellular immune responses. The immune response raised against the parasite plays an important role in resistance against the re-infection thus, strengthening the fact that ES proteins may constitute vaccine candidates. Finally, these molecules are also known to be involved in immunomodulation, signal transduction, and intracellular survival. A few in this category are cytosolic proteins but have been observed in the secretome of the parasite, including HSP70, acid phosphatase, activated protein kinase C receptor (LACK), elongation factor 1beta, and tryparedoxin peroxidase.

2.2.2.1. TSA: (Thiol-specific-antioxidant antigen) is expressed in *L. major* and is 22.1 kDa protein homologue of the eukaryotic thiol-specific-antioxidant protein, present in both the life stages - amastigotes as well as promastigotes. It has attracted the attention as the vaccine candidate because of its capability of eliciting a broad range of humoral and cellular protective immune responses in the animals infected with *L. major* [48]. Some of its important characteristics are that in the form of DNA vaccine or recombinant vaccine along

with IL-12 it induces CTL activity along with the stimulation of high titres of specific IgG1 and IgG2a antibodies and the changes in the cytokine profile can be seen as the expression is tilted towards the production of IFN-γ but decreasing the levels of IL-4 [49, 50].

TSA in the form of DNA vaccine could be a promising candidate as it induces specific immunity through CD8 response, which can suppress parasite number. A cocktail containing two leishmanial recombinant antigens (LmSTI1 [*L. major* recombinant protein homologue to eukaryotic stress-inducible protein] and TSA) induced strong protection in CL [50]. In context of cocktail vaccine Leish-111f is worth mentioning as it is composed of TSA, stress inducible protein 1 (LmSTI1), and a homolog of the eukaryotic translation initiation factor eIF4A (LeIF) with satisfactory protective responses in mice against *L. major* [51]. This recombinant vaccine has also been tested in canine model of visceral disease [52]. As a cocktail, DNA vaccine expressing complete TSA and LACK antigens has been known to be effective in *L. major* infection [53]

2.2.2.2.Sirtuins or Hst: SIR-2 (Silent Information Regulator 2) proteins belongs to a highly conserved protein family found in both prokaryotic and eukaryotic species [54]. These proteins are involved in the regulatory functions as chromatin condensation and transcriptional silencing, with histones as their physiological substrates [55]. In case of *Leishmania*, these proteins were first demonstrated in *L. major* regulating the parasite cell cycle and also acts as candidate for cell-division control marker but later studies documented their presence in the cytoplasm of *L. major* and as a part of the parasite's excreted-secreted antigens (ESAs) [56] thus, addressing a suspicious role of the SIR-2 in protection against *Leishmania*.

The sirtuins, also termed as NAD(+)-dependent deacetylases, are involved in the regulating a variety of biological processes such as gene silencing, DNA repair, longevity, metabolism, apoptosis, and development. *L. infantum* has an enzyme that belongs to this family, LiSIR2RP1, a NAD(+)-dependent tubulin deacetylase and an ADP-ribosyltransferase, essential for gene silencing in eukaryotes; it has an important role in *L. infantum* virulence and survival [57]; rSIR-2 was reported to be capable of inducing the activation and differentiation of B cells thus producing specific antibodies [58], and had a protective function in the BALB/c-mice as demonstrated by the reduction in the parasite load after *L. infantum* infection [59]. Apart from its role in regulating the gene transcription at targeted loci, SIR2 is also involved in protecting the genome from free electrons or reactive oxygen species (ROSs) as well as protect the genome and double stranded break repair through homologous recombination; a whole combination of these activities results in enhanced genome and cellular stability. *Leishmania* sirtuins from *L. major* and *L. infantum* has been demonstrated for their role in *in vitro* and *in vivo* growth of these parasites, so the protein is involved in the virulence and survival of the parasite, it can be exploited as a novel vaccine target against leishmaniasis. LiSIR2rp1(*L. infantum* SIR2 related protein) has been shown to influence the host immune system by inducing the proliferation of activated B cells consequently increasing the surface expression of CD40 and CD86 which in turn may induce T cell [58] and macrophages activation and maturation of dendritic cells thereby utilising a whole set of adaptive immune system to influence the host immune system.

2.2.2.3. Protein disulfide isomerase (PDI): Protein disulfide isomerase belongs to the superfamily of thioredoxin and is highly abundant in the lumen of the endoplasmic reticulum (ER) [60], and is the key player in assisting the folding of newly synthesized protein. It catalyzes the oxidation, reduction, and isomerisation depending on the redox environment and formation of disulfide bonds in nascent polypeptide in ER. In addition to its redox/isomerase activity PDI has been noticed to act as molecular chaperone. In the case of *Leishmania*, PDI are known to be involved in the correct folding of parasite factors which are probably required during host colonization; this protein was identified as potential virulence factor in *Leishmania* [61, 62]. PDI has been identified as a TH1 stimulatory protein as it was found to raise the levels of IgG2, a marker for TH1, with the molecular weight in the range of 89.9 to 97.1 kDa [63]. The enzyme has been known to be found in the ER, plasma membrane, cytosol and in secreted forms [64]; it is expressed and secreted at both promastigote and amastigote life stages of parasite and has been demonstrated to have high sequence homology with similar homolog from other organisms. But unlike other organisms (having four thioredoxin-like domains in PDI's and two domains with an active site) the leishmanial enzyme possesses only one active site present in a single thioredoxin-like domain [65]. PDI has also been demonstrated to have important role to play in the secretory pathway of *L. donovani* with respect to the changes in the activity of PDI.

The secreted proteins of *Leishmania* are suggested to be the important parasite virulence factors therefore, directly manipulating the activity of ER proteins can be a crucial step for targeting the parasite virulency and thus establishing it as a vaccine candidate for leishmaniasis.

PDI has been known to show the elevated levels of IgG2, is the direct measurement of the cell mediated immune response consequently raising significant levels of T_H1 type of immune response [66] along with the anti-leishmanial IgG and IgG1, thus consolidating the fact that protection against leishmaniasis is induced by a strong T cell response [67, 68]. The main marker of the T_H1-type of immune response, IFN-γ, serves as the key player in activating the macrophage microbicidal responses and other effector mechanisms. In coordination with TNF-α, it activates iNOS for the production of NO [69]. The levels of these cytokines were noticed to be down-regulated [70] in infected hamsters; whereas the expression was observed to be increased many fold in the immunized hamsters. In agreement with these observations, PDI in the form of DNA vaccine has been shown to down regulate the expressions of IL-10 and IL-4 mRNA levels in hamsters as compared with infected controls [71]. As a potential vaccine antigen, PDI has shown good prophylactic potential along with robust immune responses in vaccinated animal against visceral form of disease [72].

2.2.2.4. Secretory acid phosphatase: Whenever the parasites are in culture conditions, both promastigotes and axenic amastigotes of *L. donovani* constitutively secrete soluble acid phosphatases (SAcPs), these are the most abundant secretory proteins of this parasite with conserved antigenic epitopes. The enzyme is highly glycosylated protein of 110–130 kDa that is modified by N-linked glycans in the endoplasmic reticulum and by acid-labile phosphoglycans in the golgi complex [73]. The structural organisation is believed to be in the form of long filaments of a central chain of globular particles with a surrounding

glycocalyx, these filaments are finally assembled in the flagellar pocket. The complex purified from the culture supernatant of promastigote is composed of three components: a 100 kDa glycoprotein, a 200 kDa component and a non-covalently associated proteophosphoglycans [74].

The secreted form of acid phosphatase differs in different species of *Leishmania.* In the case of *L. mexicana*, the monomers of the enzyme assembles to form a long filament which associates non-covalently with proteo-phosphoglycans rich in lipophosphoglycan epitopes forming a polymeric phosphoglycoprotein complex and opposed to that the secreted acid phosphatase of *L. donovani* is nonpolymeric. The manipulation of the secretory products by addition of phosphoglycans is an important characteristic feature of *Leishmania*. All species (except *L. major*), secrete this enzyme in the promastigote stage of their life cycle, which is then modified by either mannooligosaccharide caps, phosphosaccharide repeats, or both [75]. These proteins may also contain Ser/Thr-rich sequences as targets for O-linked glycosidic modifications, thus, these proteins are known to play an important role in the survival of parasite survival and adaptation to extracellular stress conditions [76].

2.2.3. Vaccine targets based on Intracellular antigens—The parasite has a vast diversity of conserved intracellular protein molecules that are directly influencing the immune system after leishmanial infection, stimulating humoral responses in VL and MCL patients and raising antibodies specifically against these proteins without any cross-reactivity. This specificity is based on the location of their antigenic determinants in the more divergent regions of these parasite proteins. For that reason, these antigens posses a potential interest for diagnosis.

2.2.3.1. Heat shock proteins: The parasite has to adapt to the different environmental stresses during its life cycle. This adaptation is the part of the morphological and biochemical differentiation of parasite when itundergoes stage conversion.

Protozoan parasites of the genus *Leishmania* have a digenic life cycle; so during the transmission from the insect vector into a mammalian host they have to undergo a drastic change in environment as evident by an increase of ambient temperature by more than 10°C, a key trigger for the promastigote–to–amastigote differentiation. HSP has also been documented to play a vital role in stimulation of innate response and enhancement of adaptive immune responses, induction of dendritic cells in order to produce proinflammatory cytokines such as IL-1, IL-6, TNF-α and IL-12 [77, 78]. These proteins are also known to have adjuvant properties as a result of which they are capable of inducing strong Th1 immune response thus, solid cellular immunity [79].

Proteins of the HSP70 family are key player in the folding and assembly of newly synthesized proteins, refolding of misfolded as well as aggregated proteins, proteolytic degradation of unstable proteins, membrane translocation of proteins, and control of the activity of many regulatory proteins. HSP100 is the key player that balances between both the life stages *in vitro*; in its absence the balance shifts towards promastigote development. This is further indicated by the finding that HSP100 synthesis is upregulated all through the amastigote stage where the protein can become even more abundant than HSP70 but it has

no role in thermotolerance and viability of the parasite. HSP90 balance serves to regulate cellular homeostatic machinery, controlling both the heat stress response and morphological differentiation so overall demonstrating their role in parasite's life, as vaccine candidate they have the potential to provide considerable protection. *Leishmania* glucose-regulated protein 94 (GRP94) is member of the HSP90s family, located in the endoplasmic-reticulum is known to have a role to play in the LPG synthesis [80]. In addition to this, HSP70 and HSC70 can be further targeted to overcome the problem of drug resistance with respect to the antimonials thus, validating their importance as the candidates of interest [81].

It is worthwhile to mention about another class of parasite stress protein, the *L. major* stress-inducible 1 protein (LmSTI1), a highly conserved stress protein from yeast to humans. It has been demonstrated to raise strong proliferative response in the lymph node cells of *L. major*-infected BALB/c mice, along with the production of high levels of IFN-γ and low levels of IL-4. In the case of promastigotes, STI1 transcript levels were up regulated when a temperature shift in cultured state was done from 26°C to 37° C [82]. Furthermore, the antigenicity of the *L. major* rSTI1 was also analysed by testing with the sera from a great percentage of patients with CL, VL, and PKDL that exhibited significant reactivity toward rSTI1 [83]. Thus, it has been identified as a potent leishmanial vaccine candidate.

2.2.3.2. Cysteine proteases (CP): Proteases are ubiquitous enzymes with important metabolic functions, and are of two major types–cysteine proteases (papain like) and serine proteases (trypsin like). With respect to *Leishmania*, cysteine protease is the major protease required for the survival of parasite within the host cell as a tool for adaptation to the changing environment [84]. *L. major* contains many cysteine peptidase genes, of which enzymes of papain family have been characterised in various *Leishmania* species, which contain cathepsin L-like enzymes (CPA and CPB) as well as cathepsin B-like enzymes (CPC). *L. major* also have many other papain family cysteine peptidases, with calpain domains, that have an important role to play in calcium-dependent functions such as signal transduction, cell differentiation and apoptosis/necrosis, but their role has been demonstrated in other organisms with a rudimentary knowledge available in the context of *L. major*.

Cysteine peptidases in the form of the ubiquitin C-terminal hydrolases, small ubiquitin-like modifier (SUMO)-specific peptidase and otubain have an additional role to play in ubiquination; therefore, directs the cytosolic protein degradation thus, an important tool in the survival and adaptation of the parasite. One of the homologues of this family called as A2, is a highly conserved amastigote-specific virulence factor that induces T_H1 immune responses and confers partial protection against *Leishmania* species, therefore, making it as one of the important targets in vaccine designing [85].

The immunogenicity of the recombinant form of cysteine protease has been documented to induce T_H1 responses in patients with localized CL [86]. The PBMCs from recovered individuals produced IFN-γ on stimulation with a chimeric protein of *L. major* CPs; similar kind of data were also obtained from asymptomatically infected subjects along with the lymphocyte proliferation in addition to IFN- γ production [87].

In the case of patients with active leishmaniasis lower levels of IFN-γ, and higher levels of IL-4 and IL-10 are observed in response to the stimulation with rCPB, also the expression of IFN- γ,IL-4 and IL-10 has been noticed to be altered in oligosymptomatic cases [88]; it was also tested in the form of DNA vaccine (cpa and cpb genes) along with a booster of rCPA/rCPB adjuvated with CpG ODN and Montanide720 in BALB/c mice [89] and in canine model [90]. The immune response elicited by this vaccine was of the T_H1-type with higher ratio of IgG2a/IgG1-specific antibodies and a higher ratio of IFN-γ/IL-5; PBMC proliferation which is thought to be concordant with the presence of higher IFN-γ mRNA and less IL-10 mRNA levels [90]. Type 1 cysteine proteases has been reported as promising vaccine candidate when formulated with solid nanoparticle with specific TH1 immune responses in vaccinated mice model [91]. Moreover, there are promising finding with the use of type III proteinases in the form of DNA vaccine as a part of prime-boost strategy against *L. infantum* [92]. Thus, prime-boost strategy could be used to obtain optimal protection against *L. infantum*.

2.2.3.3. Histones: Histones are evolutionarily conserved proteins in eukaryotic organisms, located in the nucleus and are vital for the structural formation of nucleosomes, responsible for chromatin compaction when in vicinity with the DNA to form the chromatin and finally the nucleosome. *Leishmania* histone H2B protein is present in both the amastigote and the promastigote forms of the parasite and is highly conserved among *Leishmania* species. Nucleosomal leishmania histone proteins have been described as promising candidates for vaccine as these are immunodominant antigens in leishmanial infection. Histone protein synthesis is restricted to the S-phase of the cell cycle as the levels are quite low during G1,G2 and M phase of cell cycle because the genes are transcribed at high levels and mRNA's have longer half life in S-phase [93].

The role of histone H1 was demonstrated by its ability to influence the infectivity as well as the cell cycle progression, regulation and differentiation in the parasite [94] thus consolidating the role of histones as an important antileishmanial target to fish out with in near future.

Leishmania histone H2B shows relatively low level of homology with mammalian counterpart, it can be a promising candidate for vaccination because of its capability to induce the proliferation of peripheral blood mononuclear cells (PBMC) and IFN- γ levels in CL along with the capacity of induction of T_H1 response with low levels of IL-10. H2B protein could constitute the most potent vaccine in future with high level of homology at its C-terminal domain amongst species of leishmania, However, entire H2B has been found to be more potent for raising TH1 responses than either C- or N-terminal alone [95]. The potential of H2A and H2B has been documented by its leishmanicidal activity in *L. major*, *L. brazilensis* and *L. amazonensis*[96]. This fact can be further validated by the studies that demonstrate the protection conferred by the immunization of expression plasmids encoding *L. donovani* H2B, H3, and H4 in mice model [97]. In the form of DNA vaccine the mixture of the four *L. infantum* histones [98] conferred protection in BALB/c mice against the challenge of *L. major*, this was correlated with the generation of histone-specific T_H1 response in which both CD8+ and CD4+-cells were producing IFN-γ as there is a report suggesting for the fact that individual histone protein cannot be protective [99]. As a DNA

vaccine, histone proteins along with A2 (H2A, H2B, H3, H4, A2 and HSP70) has been developed as HISA70 DNA vaccine against *L. major* [100].

2.2.3.4. Ribosomal proteins: Ribosomal proteins are immunologically relevant molecules because of their evolutionary conserved nature in *Leishmania*; these molecules are important part of the leishmanial infection machinery as they are employed for dysfunctioning the immune system of the host by their capacity to manipulate cellular activities and thus, cytokine release during infection.

Acidic ribosomal proteins, also named P proteins, are constituents of the large subunit of ribosome with P2a, P2b and P0 as part of the large subunit [101]. *Leishmania* P2a and P2b are embedded with the ribosomes as complex that interacts with the P0 protein, therefore, producing a protruding stalk like structure in the ribosome. Large subunit is required for the functional activity of the ribosome and plays an important role in the elongation step of protein synthesis. The significance of these proteins as vaccine target can be suggested by the fact that antibodies were detected in the sera of dogs with VL which were specific for the *Leishmania* P2 proteins; and it does not cross-react with homologous proteins from other organisms. *L. infantum* LiP2a, acidic ribosomal protein generates strong humoral response in BALB/c mice in the absence of adjuvant and elicits IFN-γ production from splenocytes. It has been reported earlier [102] that antigenic preparation of *Leishmania* induces proliferation and IFN-γ production in peripheral blood mononuclear cells (PBMCs) from individuals, therefore documenting the immunostimulatory effect of *Leishmania* proteins may be due to the acidic ribosomal proteins [103]. The antigenic determinant region in *L. infantum* P0 protein is different from LiP2a and LiP2b protein and the antibodies to P0 protein do not cross-react with the latter.

The immunodominant nature of the P0 protein can be illustrated as the antigen was recognized by sera from both patients and animals infected with *L. infantum*. It has been known to elicit the humoral as well as the cellular immune responses in various forms (recombinant P0 or DNA vaccine of P0 gene) as demonstrated by the isotype profile of IgG1 and IgG2 and production of IFN-γ documenting for the role of T_H1-type responses [104]. Thus, LiP0 is a potential vaccine candidate when administered as DNA formulation. Recently, the LRP (*Leishmania major* ribosomal proteins) has been shown to provide protection against dermal manifestations and decreased parasite load when used in combination with CpG-ODN after challenge with *L. major* [105]. Another study documented the vaccination with *L. infantum* ribosomal protein P0 in combination with CpG –ODN as protective against CL [106]. A recombinant protein (rF14) with close homology to recombinant LiP0 has been known to impart partial protection against *L. donovani* [107]. LeIF, a 403 amino acid residues leishmanial homologue of the eukaryotic initiation factor eIF4A ; expressed in both the promastigote and amastigote stages of parasite life cycle [108], is known to be the player in a variety of molecular events such as transcription, ribosomal biogenesis, pre-mRNA splicing, RNA export, translation, and RNA degradation. LeIF has been described as a T_H1-type natural adjuvant as well as the antigen that induced an IL-12-mediated T_H1 response in the PBMC's of *Leishmania* infected individual resulting from *L. infantum*. As a recombinant antigen elongation factor has been documented as

potent TH1 stimulatory molecule with significant production of IFN-γ and IL-12 in hamsters as well as in PBMCs drawn from cured patients with VL [109].

2.2.3.5. LACK: LACK (Leishmania analogue of the receptor kinase C) is a highly conserved 36-kDa protein among related *Leishmania* species and is expressed in both promastigote and amastigote forms of the parasite. LACK and its mammalian homologue called as RACK1, belong to the trptophan-aspartate repeat protein family, are highly conserved proteins with tryptophan-aspartate motif, and are known to be located near the kinetoplast in *L. infantum*. These are involved in a variety of functions in the eukaryotes in signal transduction, RNA processing, and cell cycle control [110]. The dominant epitope has been elucidated by peptide mapping and is comprised of amino acids 156 to 173 as the major LACK peptide (LACK156–173). It is highly conserved among all *Leishmania* species required for parasite viability and for infection and parasite reproduction in the macrophage; it also plays an important role in DNA replication and RNA synthesis. LACK specific T-cells produce IL-4 and IL-10, could prevent killing of *Leishmania* parasites by infected macrophages and alters the immune response and thus favouring the parasite proliferation. Therefore, LACK can be suggested as the target for anti-leishmanial therapy, as modifying the T-cell repertoire that shows LACK expression may be helpful in controlling the course of infection. In this context, LACK, in the form of protein or DNA was capable of providing protection by against *L. major* infection as it directs the IL-4 response towards the protective $T_H1$by inducing IL-12 mediated IFN-γ production [111]. There are studies which show better performance of LACK over other vaccine antigens [112] also, as a DNA vaccine, LACK along with attenuated strains of vaccinia virus has shown varying patterns of effector immune responses. Moreover, LACK antigen along with IL-22 as a DNA vaccine has been shown to raise TH1cytokine responses and higher survival rates in mice model [113].

2.2.3.6. Other intracellular molecules: There are a series of molecules essential for the survival and adaptation of the parasite; these include sterols, and in the case of trypanosomatids, ergosterol is the key constituent for maintaining cellular structure and function. Other 24-methyl sterols that are required for their growth and viability so, can also be an excellent target for eliminating the parasite. Other molecules of this pathway include squalene synthase (SQS) required for the first committed step of sterol synthesis, squalene epoxidase, another vital enzyme required for growth and structural organization of parasite mitochondrion as demonstrated by the studies with the use of inhibitors [114]. Amongst other potential targets in ergosterol biosynthesis is the sterol methyl transferase (SMT) present only in trypanosomatids and absent from the human host. Therefore, this enzyme could be exploited as a potential vaccine target.

Glycolytic pathway is also unique in *Leishmania* as the glucose metabolism occurs in a specialised organ termed as glycosome and also the phylogenetic difference with the mammalian counterpart make it as one of the attractive targets for vaccine development. Most important glycolytic vaccine targets are glyceraldehyde-3-phosphate dehydrogenase and triose phosphate isomerase, capable of eliciting strong lymphoproliferative responses and T_H1-type cellular responses including IFN-γ and IL-12 in the PBMCs of *Leishmania*-infected cases [115].

Folate pathway has been an interesting vaccine target [116] as it has a crucial role to play in various pathways like DNA and RNA synthesis and amino acid metabolism; therefore, it is vital for growth and survival of the organism thus, enzymes such as thymidylate synthase and dihydrofolate reductase (converts dihydrofolate to tetrahydrofolate) appears to be important for the clearance of the parasite.

DNA topoisomerases are ubiquitous enzymes, major lifeline for cellular activities as replication, transcription, chromosomal segregation, genome stability etc. [117] therefore, they are essential for cell survival. These can be the potential targets for developing antileishmanial vaccine. The leishmanial P-4 protein is a nuclease (both endo- and exonuclease activities) associated with the endoplasmic reticulum of the amastigote stage of the parasite and is also involved in nucleotide excision and repair, capable of providing protection in BALB/c mice against infection by shifting the immune equilibrium towards T_H1-like response inducing increased IFN-γ (T_H1/TC1) response [118]. Therefore, it has been suggested as one of the important molecules in the intracellular survival so it can be targeted for developing anti-leishmanial therapy.

Trypanothione pathway, important regulator of oxidative stress in parasites involves the use of trypanothione which is cycled between the reduced and oxidised states by the use of trypanothione reductase. Tryparedoxin peroxidase, another important enzyme of this pathway which utilises tryparedoxin to change from its oxidised state to the reduced one for avoiding the stress faced by the parasite; therefore, trypanothione pathway is in focus as a potential candidate for vaccine development.

2.2.4. Vaccine targets based on Membrane/Surface Antigens—*Leishmania* parasites show different surface molecule composition during their life cycle. Both procyclic and metacyclic promastigotes are covered by glycoproteins and other glycosylated molecules, which are anchored to the surface membrane by glycosylphosphatidylinositol (GPI) constituting the vital structural component of the parasite.

2.2.4.1. KMP11: Kinetoplastid membrane protein 11 (KMP-11) is one of the most abundant 11-kDa surface membrane glycoprotein found in non-covalent association with LPG molecule. This 11-kDa protein, encoded by a 276 bp open reading frame is a dominant membrane glycoprotein expressed throughout the promastigote and amastigote forms of *Leishmania* parasites but the surface expression increases in amastigotes and in metacyclic promastigotes during metacyclogenesis [119].

At the structural level, it has the potential to form a hairpin-like structure with both hydrophilic and hydrophobic surfaces so it is thought that KMP-11 facilitates a strong interaction with lipid bilayers. The protein has been known to have vital function in the immunology of leishmaniasis and suggested as a potential vaccine candidate because of its ability to induce potent immune response.

However, KMP-11 is the only antigen to be recognised by the sera of asymptomatically infected [119], in addition, it is also capable of inducing IFN-γ from PBMC of cured cases in Kenyan population [120]. In the form of DNA vaccine, KMP-11 is known to be effective

against both antimony-sensitive and -resistant virulent *L. donovani* strains [121]. in another study in Sb-resistant and susceptible parasites role of IL-4 and NO on use of KMP-11 as a DNA vaccine has been elucidated [122]. IFN-γ and TNF-α, the major cytokines of the immune pathway for killing the parasite work in concert with the iNOS to produce NO and thus, potentiating a strong immune response to clear off the parasite therefore, proving the role of KMP-11 as stimulator of T-cell proliferation and immune response regulator.

In an effort to establish KMP-11 as vaccine candidate, other major studies included the use of *Toxoplasma gonadii*-mutant (ts-4) expressing KMP-11[123], peptide pulsed-DC in adjunct to CpG-ODN than using peptide pulsed-DC alone against visceral infection [124]. Moreover, in the form of DNA vaccine KMP-11 along with IL-12 has also been shown to provide protection against *L. major* comparable to DNA-vaccine KMP-11 as in *L. donovani/L. infantum* [125] has raised new hope for further explorations considering this molecule as an attractive candidate.

2.2.4.2. LPG: *Leishmania* cell surface has a dense covering of glycocalyx, surface molecules are attached by glycosyl-phosphatidylinositol (GPI) anchors or GPI-related molecules such as the gp63 and proteophosphoglycans (PPGs). In addition there are abundant GPI-anchored glycosylinositolphospholipids (GIPLs) that do not attach protein to them but form a densely packed layer onto parasite surface. LPG is the most important and complex glycophospholipid that consists of type-2 anchor (Man_1–3Man_1–4GlcN_1–6PI motif) attached to a phosphosaccharide-repeat domain, which undergoes species-specific side-chain modifications with a capping of neutral oligosaccharide [126].

LPG has been known to play an important role in survival of the parasite in the adverse macrophage environment by resisting the parasite from the hydrolytic enzymes as well as the oxidative stress imparted by the host machinery also inhibiting the phagolysosomal fusion and macrophage activation after the infection and protecting the parasite from the host complement system [127].

In context of the immunological responses, LPG from metacyclics induced an increased production of IFN-γ and TNF- α by producing iNOS [128] thus mediating leishmanicidal effector mechanism in the macrophage and host-protection against *L. major* infections in the mice model [129].

In the form of vaccine candidate against leishmaniasis it has been used in combination with polyacrylic acid without any toxic effects in the murine model emphasising for the need to be studied further for developing a potential vaccine [130]. LPG along with BCG has been shown to raise TH1 immune response in mice as well as hamster model [131]. LPG has also been established as an important target for the development of transmission blocking vaccine [132].

Another molecule is a 36-kDa glycoprotein called as FML (fucose-mannose ligand), is the human marker of the disease, that inhibits the penetration of promastigote [133] as well as the amastigote [134] in the macrophages in a species specific manner in mice model [135]. It is documented as a protective antigen in mice in the form of DNA or native recombinant

vaccine. It is amongst the licensed canine vaccine that has been successful in phase III trial [136] commercially available as Leishmune, with immunogenic, immunoprophylactic and immunotherapeutic potential in hamsters, mice and dogs [137, 138].

2.2.4.3. gp63, gp46/PSA,HASPB: Glycoprotein 63, or leishmanolysin (or major surface protease), is a zinc metalloprotease found on the surface of kinetoplastids helpful in mediating parasite entry into the macrophages, enhancing phagocytosis and survival within the macrophage. Moreover, the proteinase activity of gp63 plays an important role in controlling the process of complement activation, so in a way it provides the parasite with the resistance to cope up with complement-mediated lysis [139]. The parasites are efficient enough for exploiting the macrophage signalling especially the JAK-STAT pathway, the key regulator of the IFN-γ signalling [140]. In the form of polytope DNA vaccine, gp 63 induced strong T_H1 responses and much stronger cytotoxic responses than the single-antigen gp63 DNA vaccination [141]. Potency was better, with superior efficacy, and durability than protein (recombinant or native) and heterologous prime-boost (HPB) preparation. It had protective effects against the infection challenges [142]. Therefore, GP63 can be documented to be an important molecule in the survival and sustenance of the parasite so it can be suggested as a candidate for vaccination against *Leishmania* infection.

Promastigote surface antigen (PSA, also known as GP46) is highly abundant surface glycoinositol phospholipid anchored glycoprotein expressed at both the life stages of the parasite [143]; but the level fluctuates from exponential phase to stationary phase in parasites, thus, suggesting for its role in metacyclics. PSA has been known to be an important tool for parasite as it is responsible for imparting resistance to complement mediated lysis. It is also capable of eliciting a protective immune response in combination with an adjuvant; the cells showing reactivity against PSA-2 are known to produce high levels of IFN-γ, thus, conferring natural immunity to leishmaniasis through T_H1 response. The most recent ongoing project is RAPSODI, aiming to design a pan-Leishmania vaccine is a PSA –based human vaccine targeting all forms of leishmaniasis.

The *Leishmania*-specific hydrophilic acylated surface proteins (HASPs), known by its type HASPB, are expressed on the plasma membrane of infective parasite stages only (metacyclics and amastigotes) and suggests for some (inter- and intra-specific) variations, mainly in the repetitive amino acid domains [144]. The HASPs undergoes co-and post translational modifications that are essential for their membrane trafficking and localisation at the plasma membrane [145]. The SHERP (small hydrophilic endoplasmic reticulum associated protein) a 6.2 kDa peripheral membrane protein which is found to be localised in cytosolic face of the ER and the outer mitochondrial membrane in the metacyclic stage of parasite, interaction of these two proteins with the lipids in the membrane is well known but the function is not yet clear but their ability to act as a vaccine candidate has been well documented.

2.2.5. Vaccine targets based on salivary proteins—*Leishmania* is recruited inside the host together with saliva of the sand fly during the blood meal. Saliva has been known to contain a repertoire of pharmacologically active molecules that contributes towards the establishment of the infection and assisting the sand fly to acquire the blood meal, therefore,

considered as highly immunogenic molecule in humans [146], canids [147] and mice as a part from the salivary proteins it may also contain some *Leishmania*-derived antigens that are potentially inducing the protective immunity in the host. Amongst the important targets is sand fly salivary gland homogenate (SGH) that has been proved to be protective in mice against *Leishmania major* [148], narrowing down the research to get the exact target demonstrated for the two major salivary proteins – maxadilan and PpSP15; maxadilan from *Lutzomyia longipalpis*, where the immunity against the molecule leads to the neutralisation of exacerbatory infection [149]; maxadilan has also been known to influence the cytokines associated with T_H2 responses (IL-6, IL-10, and TGF-β) but known to downregulate T_H1 cytokines (IL-12p70 and TNF) and nitric oxide (NO) [150]. PpSP15 from *Phlebotomus papatasi*, capable of providing immunity in mice (wild-type and B-cell deficient) models suggesting for the fact that cellular immunity to PpSP15 is major weapon in conferring immunity [151]; promastigote secretory gel (PSG) or chemically defined synthetic glycovaccine from *L. mexicana* raises significant protection against the bite of infected sand flies but neither of the two (PSG or glycovaccine) were effective in providing immunity against artificial needle challenge [152]. There are other salivary proteins as salivary protein (LJM19) [153] that are the key players deciding the fate of infection. Therefore, it is worthwhile to consider the salivary component as vaccine target because of the immunogenic potential that leads to the lymphocyte infilteration, IFN-γ and IL-12 production [154].

There are other views about saliva based components that suggests for the fact that instead of vector saliva, parasite secreted proteophosphoglycans (including promastigote secretory gel), are responsible for enhanced disease following sand-fly-transmitted versus needle infection and are viable vaccine targets.

3. Expert commentary and five years view

There is huge demand for the potential vaccine in field of leishmaniasis as the existing therapeutic measures are threatened by their toxicity and drug resistance and if treated, various forms of the disease may cause lifelong disfiguring and scarring lesions so, it is better to prevent the disease rather than treating it. Therefore, prophylactic vaccination will be an efficient step in controlling the disease but in this regard despite ongoing research there is no licensed vaccine available in the market till date but the genomic studies have really paved the way for a wide range of studies in targeting the parasite and have been a ray of hope for the scientific community.

Considering the future development in this field it would be easier to say that polyprotein and DNA vaccines (plasmid DNA encoding *Leishmania* antigens) document for a novel approach of vaccine development against leishmaniasis as seen in Leish-111f and LiESA/QA-21 of which LiESA (CaniLeish) is the first licensed vaccine developed in Europe and Leish-Tec developed in Brazil for canids. DNA vaccine because of its intrinsic adjuvant properties in addition to the capability of inducing both humoral and cellular arms of immune responses has led to the long lasting immunity. These do not face the drawbacks of second generation vaccines in HIV-coinfected cases as they are known for raising cell-mediated immune responses, and can be used even in immune compromised cases; other

future aspects in the field of leishmaniasis vaccine includes the use of dendritic cells (DC) as a vector for delivery of leishmanial antigen being the encouraging force for the scientists. It is worthwhile to mention about the manipulations that can be done in *Leishmania* genome in order to create genetically modified parasites (either by deleting or introducing genes) for producing live- attenuated vaccine thus, providing a new alternative in field of development of new generation vaccine against leishmaniasis.

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Abbreviations used

VL	Visceral leishmaniasis
CL	Cutaneous leishmaniasis
MCL	Mucocutaneous leishmaniasis
PKDL	Post kala-azar dermalleishmaniasis
NTD	Neglected tropical disease
IFN-γ	Interferon-gamma
IL-10	Interleukin-10
DC	Dendritic cells
GLASE	Glucopyranosyl lipid adjuvant-stable emulsion
iNOS	inducible nitric oxide synthase
NO	nitric oxide
GRP94	Glucose regulated protein94
PBMC	Peripheral blood mononuclear cells
SQS	Squalene synthase
SMT	Sterol-methyl transferase

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Key issues

1. The drawbacks associated with the existing anti-leishmanial drugs have forced the need for a vaccine; the feasibility is fuelled by the fact that recovered subjects are immune to reinfection.
2. The availability of genome sequence of *Leishmania* has provided whole array of information regarding the molecular targets which can be exploited for the purpose of vaccine development.
3. Leishmanization although banned due to safety concerns has made a remarkable contribution in advancement of the vaccine designing and addressing various issues concerned with the vaccine development.
4. Leish-111f or LiESA/QA-21 has created positive impact in field of development of a new vaccine.
5. Some of the licensed vaccines for canine have increased the interest for promoting the trials in human models.

Table 1

First generation (Killed and live/live-attenuated) vaccine trials

Target antigen	Target disease/Protection	Vaccine type	Experimental model	Reference
L. major	CL/Protection	Live promastigotes	Mice	[155]
L. infatum/L. donovani	VL/Protection	Long term culture with gentamycin	Mice	
L. braziliensis	CL/Protection	Avirulent *L. brazieliensis*	Mice	[156]
L. major	CL/Protection	DHFR-TS-Knock-out promastigote	Mice	[157]
L. major	CL/Protection	*lpg*2-mutant promastigote	Mice	[158]
L. infantum	VL/Protection	SIR2 deficient promastigotes	Mice	[159]
L. mexicana	CL/Protection	Cysteine-protease deficient promastigote	Hamster	[160]
Autoclaved *L. amazonensis*	CL/Protection	Killed parasite+BCG	Human	[26]
Merthiolate-dirupted *L. brazieliensis*	VL/Partial protection	Killed+BCG	Dogs	[161]
Autoclaved *L. major*	CL/Protection	Killed+CpG/rIL-12 DNA vaccine	Mice	[162
Autoclaved *L. major/L. donovani*	VL/Protection	Killed+ALM-BCG	Hamster	[163]
Killed *L. brazieliensis*	CL/-	Killed+Saponin	Dogs	[163]
Autoclaved *L. amazonensis*	CL/Protection	Killed+BCG	Human	[25]

Table 2

Second and third-generation vaccine trials with potent vaccine targets

Vaccine targets	Subcellular location	Type of vaccine	Experimental model	References
HSP/HSC/LmSTI-1	Cytosol	DNA Native antigen+MPL/ALD	Mice	[164, 165]
gp63	Memberane	DNA vaccine(DNA+pCMV)	Mice	[166, 167]
H1	Nucleus	Recombinant protein	Mice	[168]
CPa+CPb	Cytosol	Recombinant protein	Dog	[169]
LACK	Cytosol	Recombinant protein	Mice	[170]
P0	Ribosome	Recombinant protein	Mice	[106]
LACK	Cytosol	DNA vaccine	Mice	[171]
KMP-11	Memberane	pcDNA-LIC	Hamester	[122]
A2	Cytosol	Recombinant protein	Mice	[172]
LACK	Cytosol	pcDNA-3+vaccinia virus	Mice	[36]
CPa or CPb,CPa+CPb	Cytosol	pCB6	Mice	[173]
H2A-H2B and H3,H4	Nucleus	DNA vaccine	Mice	[174]
LACK	Cell surface	DNA vaccine	Mice	[175]
HASPB	Cell surface	Recombinant protein	Mice	[176]
LPG	Cell surface	native antigen+BCG	Mice	[177]
Hb-receptor	Flagellar pocket	DNAvaccine	Mice and hamster	[43]
LiP0- PB	Cytosol	pcDNA3	MiceMice	[178]
CPc-PB	Cytosol	DNA vaccine	Hamster Mice and human	[92]
KMP-11	Cell surface	DNA vaccine		[122]
Leish111-f	-	Recombinant poly protein		[39]
LiESA/QA-21	Excreted/secreted protein	Recombinant protein	Dog	[179]

- PB- Prime- boost