Scholarly Research Exchange

Volume 2009 • Article ID 173039 • doi:10.3814/2009/173039

Review Article

Searching Genes Encoding *Leishmania* Antigens for Diagnosis and Protection

Manuel Soto,[1] Laura Ramírez,[1] Miguel A. Pineda,[1] Victor M. González,[2] Petter F. Entringer,[3] Camila Indiani de Oliveira,[3] Ivan P. Nascimento,[3] Ana P. Souza,[3] Laura Corvo,[1] Carlos Alonso,[1] Pedro Bonay,[1] Claudia Brodskyn,[3] Aldina Barral,[3] Manoel Barral-Netto,[3] and Salvador Iborra[4]

[1] *Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
[2] *Departamento de Bioquímica-Investigación, Hospital Ramón y Cajal, 28034 Madrid, Spain*
[3] *Centro de Pesquisas Gonçalo Moniz, Fundação Oswaldo Cruz (FIOCRUZ), Rua Waldemar Falcao, 121 Candeal, 40296-710 Salvador, BA, Brazil*
[4] *Unidad de Inmunología Viral, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Crta. Majadahonda Pozuelo Km 2, Madrid 28220, Spain*

Correspondence should be addressed to Manuel Soto, msoto@cbm.uam.es

Received 12 January 2009; Revised 17 April 2009; Accepted 14 May 2009

Leishmaniases are a wide spectrum of parasitic diseases caused by the infection of different species of the genus *Leishmania*. Currently, these diseases are one of the most neglected diseases threatening 350 million people in different countries around the world. Thus, these diseases require better screening, diagnostics and treatment. An effective vaccine, that is not currently available, would be the best way to confront leishmaniases. In the past 20 years the molecular characterization of *Leishmania* genes encoding parasite antigens has been carried out. In this review we summarize the most common strategies employed for the isolation and characterization of genes encoding *Leishmania* antigens. To provide a collective view, we also discuss the results related with diagnosis and protection based on different recombinant DNA-derived *Leishmania* products.

1. Introduction

Leishmaniases comprise a complex group of diseases caused by several intracellular protozoa of the genus *Leishmania* that infects macrophages from a variety of mammals including human and dogs. These parasites possess a digenetic life cycle and they develop as promastigotes in the gut of the blood-sucking phlebotominae sandflies and as intracellular amastigotes in macrophages of their vertebrate hosts. Depending largely on the species of the parasite and the immunocompetence state of the human host, the disease spectrum ranges in severity from cutaneous (CL), post-kala-azar dermal (PKDL) and diffuse cutaneous (DCL), to mucocutaneous (MCL) and visceral leishmaniasis (VL). These infections are endemic in 86 tropical and subtropical countries around the world, accounting for at least 75.000 deaths per year [1]. Canine viscerocutaneous leishmaniasis (VCL) is an important emerging zoonosis in countries around the Mediterranean basin, in the Middle East, and in Latin America [2]. This severe form of the disease is caused by *Leishmania infantum* in the Mediterranean area, Middle-East, and Asian countries and by *Leishmania chagasi* in Latin America being wild canids and domestic dogs the major reservoir, playing central role in the transmission to humans [3]. The outcome of infection is determined by interactions between the host immune system and the different parasite species, yet the pathogenesis of leishmaniasis remains unclear and the understanding of the mechanisms involved in the immune response to *Leishmania* in humans and dogs is still limited. Generally, protective immunity is associated with a classical cell-mediated immune response that induces macrophage activation by T cells-derived cytokines, while nonhealing disease is associated with the generation of strong humoral responses [4, 5].

Conclusive diagnosis of leishmaniasis depends on the detection of amastigotes after staining of bone marrow or splenic aspirates from visceral cases, or biopsy samples taken

from the infected tissues in cutaneous or mucocutaneous patients [6]. The detection of promastigotes by culturing the biopsy samples in a suitable medium can also be employed for diagnosis [6, 7]. The presence of anti-*Leishmania* specific antibodies during infection has allowed the development of serologic test including immunofluorescent antibody test (IFAT), western blot, immunochromatographic test, and enzyme-linked immunosorbent assay (ELISA) [7]. Sensitivity and specificity of these assays depend on the antigen preparation employed since some of the parasite antigens exhibit cross-reacting epitopes shared by other pathogens. As an strategy to develop specific serodiagnostic test for leishmaniasis, the genes encodings for some parasite antigens were isolated in order to obtain recombinant molecules suitable for serodiagnosis [8].

Different strategies have been employed for the generation of vaccines against *Leishmania* but, to date, there is no vaccine against this parasite in routine use. The history of *Leishmania* vaccines has been reviewed by different authors [4, 9–11]. Progress toward vaccine development has included human trials using live vaccines with *Leishmania major* promastigotes (parasites that cause self healing skin ulcers) performed in the 1980s. Although a high percentage of successful lesion reduction was observed [12, 13] the use of these live vaccines had so many secondary effects that made difficult the standardization of these approaches. Vaccines based on killed promastigotes were also analyzed. The results from several clinical trials using whole parasite antigens showed little or no protection [9, 10]. For the past 20 years, DNA cloning and characterization of genes encoding parasite proteins suitable for the development of defined vaccines have been carried out.

For the development of diagnostic tools as well as second-generation vaccines based on recombinant products (recombinant proteins and/or DNA vaccines) the genes coding for parasite antigens should be cloned and characterized. The first approach for the characterization of *Leishmania* antigens was the biochemical purification of some parasite membrane fractions or proteins secreted different live stages of the parasite (usually promastigotes). *Leishmania* secreted factors as well as proteins located in the parasite surface are the most important in the establishment of the infection, either during the first contact of the parasite with the host cell, or interfering with immune cells functions, like cytokine production, antigen presentation, or cell activation. As many of these molecules are complex antigen mixtures, their role in diagnosis and protection is not described in detail in this review. However, it is worth mentioning that several protein fractions were used to assess their immunogenicity. Further examples are FML, LiESAp-MDR, Ric-1, and Ric-2. FML stands for Fucose-Mannose Ligand and it is a membrane-enriched preparation of *L. donovani* [14] that can be employed for serodiagnosis of human and canine VCL [15, 16]. FML was patented in Brazil as Leishmune and it is being used commercially for canine vaccination [17]. The major immunogenic component of the FML extract is a glycoprotein of 36 kDa (LdGP36) [18]. To date, this protein was not obtained as a recombinant product. The combination of naturally excreted/secreted antigens, purified from culture supernatant of *L. infantum* promastigotes (LiESAp) with muramyl dipeptide (MDP) as adjuvant, conferred protection to dogs experimentally infected with *L. infantum* [19]. A double blind trial was performed in naturally infected dogs in France with LiESAp, showing a significant decrease in the incidence of infection two years after the vaccination [20]. Ric-1 and Ric-2 are also protein fractions secreted by *L. infantum* promastigotes, Ric-1 contains high molecular weight excreted proteins and Ric-2 the low molecular weight ones. These two proteins fractions were able to induce different immune responses, mainly by the modulation of the Th1/Th2 cytokine balance [21]. Immunization with Ric-1 and Ric-2 resulted in a reduction of 50% and 67% in the parasite burden of the spleen from infected mice, respectively [22].

The most common assay for the isolation of genes coding for surface or secreted proteins was the generation of genetic probes designed on the basis of the aminoterminal aminoacid sequence obtained after their biochemical purification. These genetics probes were employed for the screening of genomic libraries constructed in bacteriophage lambda or cosmid vectors [23–25] (Figure 1). Alternatively, the sera from mice immunized with the purified secreted fractions were employed for the immunoscreening of cDNA or genomic expression libraries (since *Leishmania* protein coding regions are almost never interrupted by introns [26, 27]) to obtain the genes encoding for the most immunogenic proteins contained in these fractions [28–30] (Figure 1).

After *Leishmania* infection, humoral responses against different parasite antigens are elicited and significant antibody levels are detected in the sera from patients and dogs suffering the disease (reviewed in [7]). For that reason, the immunoscreening of parasite expression libraries (cDNA or genomic) with the sera from dogs suffering VCL [31–36], or human patients with VL [37, 38] or with MCL [39, 40] was the most common strategy employed in the identification and isolation of *Leishmania* antigen coding genes. Also, the sera from experimentally infected mice were employed for immunoscreening purposes [41]. Usually, the antigens isolated after these screenings were intracellular proteins. In addition, many of them can be considered as members of conserved families (reviewed in [42, 43]) (Figure 1).

Some other experimental approaches were employed for the characterization of *Leishmania* antigens. Persistent immunity against *Leishmania* infection is mediated predominantly by $CD4^+$ T cells of the Th1 phenotype [44–46]. For this reason, the characterization of library clones that stimulate interferon-gamma (IFN-γ) production in T cell clones established from *L. major*-infected mice was an alternative procedure for the isolation of parasite antigen encoding genes [47, 48]. Also, T cell clones were established from Montenegro (DTH) skin test positive adults residing in regions endemic for VL [49]. Interestingly, many of the genes identified using this methodology were the same genes identified after immunoscreening of expression libraries with the sera from infected human or dogs (Figure 2).

Mice immunization with naked DNA composed of mammalian expression vectors recombinant for heterologous genes induces cellular (mainly $CD4^+$ Th1 and $CD8^+$

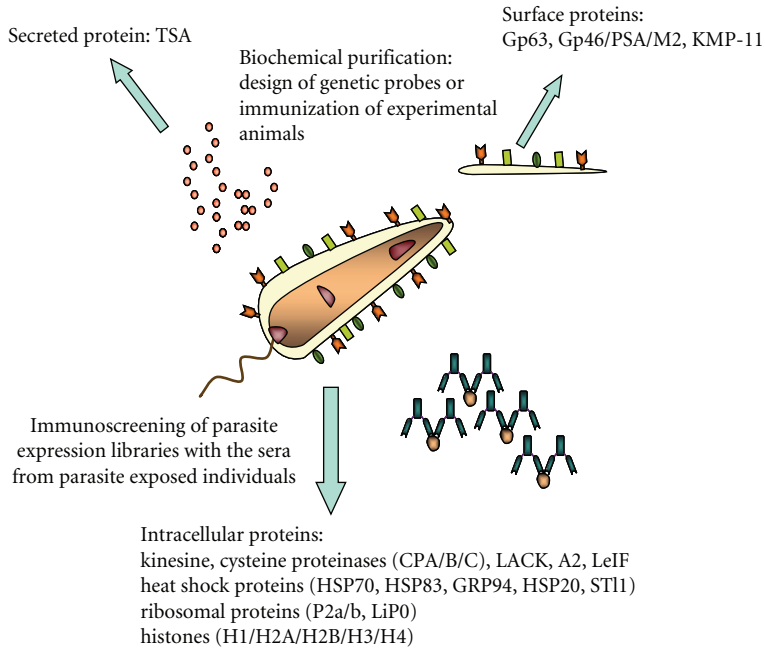

Figure 1: The most common approaches employed for the isolation of genes encoding *Leishmania* antigens.

Table 1: Genes encoding *Leishmania* secreted/excreted antigens.

Thiol-specific antioxidant	(TSA)	*L. major* [28]
Sirtuin-2	(SIR-2)	*L. major* [58]

responses) and humoral responses, that are protective against several pathogens (reviewed in [50, 51]). Immunization with genomic expression libraries (ELI) of *Leishmania* induced protective responses against parasite infection in mice [52]. Thus, ELI emerged like an alternative approach for the characterization of genes encoding parasite protective antigens. For that purpose, sequential fractions of *Leishmania* libraries constructed in eukaryotic expression plasmids were isolated on the basis of the induction of protective immunity and the genes composing these fractions were identified [53, 54] (Figure 2).

Finally, the search for genes overexpressed in the infective form (metacyclic promastigotes) or in the amastigote stage allowed the characterization of different genes coding for parasite antigenic proteins [55–57].

In this work the strategies employed for the isolation of genes encoding parasite antigens were reviewed. We summarize the results related with diagnosis and protection based on different recombinant DNA-derived *Leishmania* products.

2. Diagnostic and Vaccines Based on Secreted and Excreted Antigens

Two parasite genes encoding secreted proteins have been characterized (Table 1).

The *Leishmania* homolog of the eukaryotic thiol-specific-antioxidant (TSA) proteins was identified in 1998 by Webb et al. [28]. This protein was located in the culture filtrate material from in vitro cultivated *L. major* promastigotes (CFP). The CFP was highly antigenic and immunogenic in the experimental murine leishmaniasis model [47]. To identify immunogenic components of the promastigote CFP, serum samples from CFP-vaccinated BALB/c mice were used to screen an *L. major* cDNA expression library. The cDNA encoding *L. major* TSA was isolated and expressed as a recombinant protein in bacteria (rTSA) [28]. Immunization of the recombinant protein using interleukin-12 (IL-12) as adjuvant resulted in the protection against *L. major*-infection [28]. Parasite TSA was antigenic and immunogenic not only in the murine system but also in *Leishmania* infected humans. Thus, the sera from some CL (12/27) and VL (12/28) patients contain significant titres of antibodies against rTSA [28]. Also, this antigen elicited in vitro proliferative responses from peripheral blood mononuclear cells (PBMCs) obtained from these patients [28]. The presence of specific anti-TSA antibodies in the sera from dogs with VCL was recently demonstrated [59] (see Table 2).

The protein SIR-2 belongs to a highly conserved protein family found in both prokaryotic and eukaryotic species named Hst proteins or sirTuins [60]. Historically, the biological importance of SIR-2-related proteins was attributed to chromatin condensation and transcriptional silencing, having the histones as their physiological substrates [61]. However, other localizations were documented among the diversity of SIR-2 homologues in different organisms. *Leishmania* SIR-2 cDNA was identified in 1996 by immunoscreening of an *L. major* cDNA library with the sera from a mouse immunized with *L. major* antigens that interact with glutathione [58] but subsequently it was found in other *Leishmania* species with a high degree of homology [62]. The interest of the protein was related to its possible role in the regulation of the parasite cell cycle and as a putative

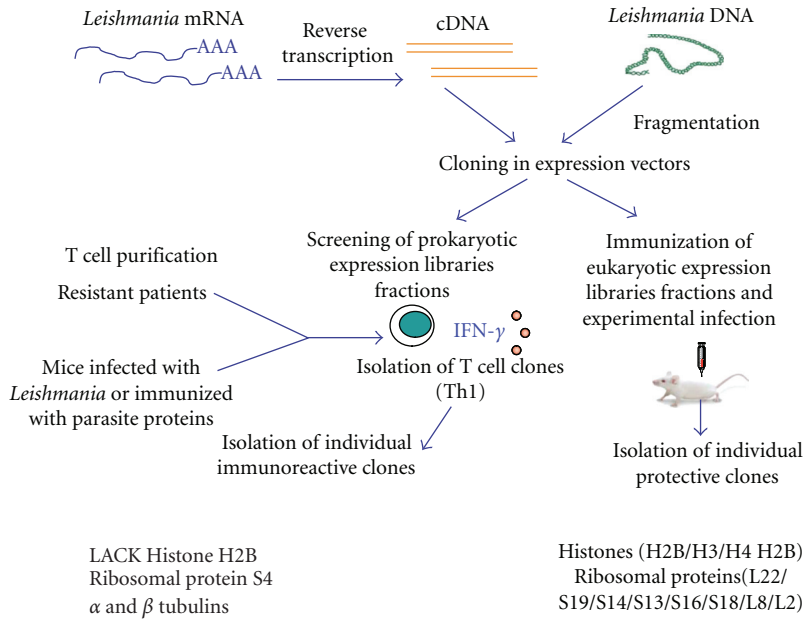

Figure 2: Other approaches employed for the isolation of genes encoding *Leishmania* antigens.

candidate for a cell-division control marker, a biological function that possessed its homologous protein in yeast. However, SIR-2 was shown two years later to be located in the cytoplasm of *L. major* and also that the protein is among the parasite excreted-secreted antigens (ESAs) [63]. These findings addressed the SIR-2 biological role in *Leishmania* to an unknown role, rather than the gene expression regulation.

The *L. major* SIR-2 protein was obtained as a recombinant protein expressed in bacteria [63]. rSIR-2 was able to induce the activation of B cells from normal BALB/c mice and its injection induced B-cell differentiation and production of specific antibodies through a T-cell the independent mechanism [64]. Due to all these observations, it was postulated that the SIR-2 protein may contribute to the establishment of infection by the induction of humoral responses [42]. However, it was also postulated that the presence of SIR-2 specific antibodies induced by immunization with the rSIR-2-can have a protective role since BALB/c-vaccinated mice presented a reduction in the parasite load after *L. infantum* infection [42].

The use of the *L. major* rSIR-2 as antigen demonstrated that this protein is highly antigenic during natural canine infections both in symptomatic and asymptomatic infected dogs, showing a preferential IgG2 production [65]. The rSIR-2 was also recognized by the sera from children with VL caused by *L. infantum* infection [66] (see Table 2).

3. Diagnostic and Vaccines Based on 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) forming the glycocalix which is almost completely absent from amastigotes [67, 68]. The main surface molecule of promastigotes is lipophosphoglycan (LPG), although some other surface molecules were described. Four different *Leishmania* genes encoding surface proteins have been characterized (Table 3). In this section we summarize the role of these membrane proteins in the development of diagnostic tools as well as vaccines (see Table 4).

The *Leishmania* proteinase GP63 is one of the most abundant surface-exposed proteins on parasite promastigotes [67, 69]. GP63 was identified and purified in 1985 [70]. The most commonly used name, GP63, is derived from glycoprotein of 63 kDa, even though isoforms with different molecular weights were described in many *Leishmania* species [71]. The first gene encoding GP63 was isolated from *L. major* [23]. A synthetic oligonucleotide probe based on the aminoterminal protein sequence of purified GP63 was employed in the screening of an *L. major* genomic library [23]. Since then, the genes encoding this protein were characterized in many other *Leishmania* species (see [71] for a review). The conservation of the coding sequence of GP63 amongst diverse species of *Leishmania* provides further support for the importance of GP63 during the life cycle of the parasite [23, 70, 72–74].

Several authors analyzed the antigenicity of the GP63 protein. The use of native *L. donovani* GP63 as antigen in ELISA seems to distinguish an ongoing from a past VL infection [75]. *L. chagasi* and *L. donovani* GP63 were obtained as recombinant proteins expressed in bacteria and were employed in ELISA assays [76]. The sera from the most acute VL patients showed a high reactivity against both recombinant proteins while sera from other forms of leishmaniasis (CL and MCL) and from Chagas' disease patients showed very low reactivity [76]. *L. infantum* rGP63 was recognized by the sera from dogs naturally infected with this parasite [77]. These results indicate that rGP63 might be

Table 2: Summary of secreted excreted antigens regarding diagnosis and vaccination.

Molecule	Diagnosis	Vaccination	
	Presence of antibodies documented in	Animal model	Immunization mode
TSA	Human VL and CL [28] Canine VCL [59]	Mice CL	rTSA + IL-12 [28]
SIR-2	Human VL [66] Canine VL [65]	Mice VL	rSIR-2 [42]

Table 3: Genes encoding *Leishmania* surface antigens.

Glycoprotein of 63 kDa	(GP63)	*L. major*
Glycoprotein of 46 kDa	(GP46/M2/PSA)	*L. major*
		L. amazonensis
Kinetoplastid membrane protein	(KMP-11)	*L. donovani*
Hydrophilic surface protein B1	(HASPB1)	*L. major*

a useful constituent of a defined serologic test for the visceral forms of the disease. Techniques employing the GP63, other than ELISA were also studied for *Leishmania* diagnosis. The presence of a major antigen of 55 kDa was observed in an immunoblot prepared with the immune complexes (ICs) isolated from a kala-azar patient serum when it was incubated with an anti-GP63 antibody. This recognition suggested that 55 kDa antigen and GP63 had common antigenic epitope(s) and the authors concluded that the former antigen was processed from GP63. In summary, the identification of parasite antigen (55 kDa) in ICs of kala-azar patients sera may be useful in the development of a serodiagnostic assay of VL [78, 79]. Antibodies against GP63 were also employed in an indirect immunofluorescence assay (IFA) to identify amastigotes from lesion fluid aspirates [80].

The efficacy of vaccines based on GP63 was firstly tested by the administration of a native purified GP63 reconstituted in liposomes. These liposomes induced appreciable levels of protection depending of the route of immunization in different murine models of CL [81, 82] or VL [83, 84]. Similar results were found by Jaafari et al. studying the ability of the *L. major* rGP63 expressed in bacteria [85, 86]. The recombinant protein lacking the sugar molecules present in the native protein-induced protection against *L. major* infection in susceptible BALB/c mice when it was administered entrapped in liposomes in the absence [85] or in the presence of CpG oligodeoxynucleotides (CpG ODN) [86]. In these works it was shown that immunization of rGP63 alone conferred a partial protection while entrapment of rGP63 in liposomes significantly increased the rate of protection, particularly when coadministered with CpG ODN, inducing high Th1 responses. Taken together, the results indicate that liposomes may be used as a suitable adjuvant for the development of vaccine and that coencapsulation of CpG ODN in liposomes improves the immunogenicity of the GP63 *Leishmania* antigen.

Recombinant *Salmonella typhimurium* [87] or bacille Calmette-Guérin (BCG) [88, 89] expressing *Leishmania* GP63 were also tested for the development of vaccines in murine experimental models. Spleen cells from CBA mice orally immunized with *S. typhimurium* expressing rGP63 developed antibody and proliferative T cell response to *L. major*. The activated T cells are mainly $CD4^+$ and secrete IL-2 and IFN-γ but no IL-4 [87]. These mice did not develop lesions after infection with *L. major* [87]. Mice immunization with recombinant BCG producing GP63 elicited significant protection against a challenge with *L. major* [88] and a strong protective response against challenge with *L. mexicana* promastigotes or amastigotes [89]. Finally, immunization of a eukaryotic expression plasmid containing a cDNA coding for *L. major* GP63 in BALB/c mice induced Th1 responses specific for the parasite GP63 and the mice showed a partial protection against *L. major* infection [90].

Defined vaccines based in GP63 peptides were also tested. Thus, wo peptides representing predicted T-cell epitopes of GP63 were tested on a murine model of CL. Either subcutaneous or intraperitoneal immunization in saline with a peptide representing GP63 amino acids 467–482, significantly protected CBA mice against the development of severe cutaneous lesions only when the peptide was intrinsically modified by covalently adding of a lauryl-cysteine moiety (LC-p467) to its amino terminus during synthesis. In marked contrast, administration of p467 alone, cysteinyl-p467, or GP63 protein in saline resulted in some disease exacerbation. Splenic cells of LC-p467, immunized mice stimulated in vitro with LC-p467 displayed strong proliferative responses and secretion of IL-2, IFN-γ and GM-CSF (but not IL-4 and IL-10) suggesting that immunization with the lipopeptide induced Th1 like cytokine responses associated with cell-mediated immunity [91]. In anther approach, Tsagozis et al. tested the efficacy of an experimental vaccination in murine models of CL using bone marrow-derived dendritic cells (BMDCs) pulsed with a GP63 peptide (positions 154–169) [92]. Antigen-specific Th1 immune responses were generated that correlated to a reduction in lesion formation and parasite burden. These findings suggest that vaccination with BMDCs pulsed with defined peptides could be a strategy against infectious diseases [92].

It was described the existence of another family of glycoprotein in the promastigote membrane of different *Leishmania* species (except *L. braziliensis* [93]) namely PSA-2 (for promastigote surface antigen 2 [30]), GP46 (because of its molecular weight, or M2 (because of its reactivity against a monoclonal antibody, namely, M2 [94]). *L. major* GP46 coding gene was isolated after immunoscreening of a genomic expression library with the polyclonal antiserum of a mice immunized with surface and integral membrane proteins of the parasite [30]. Purified *L. amazonensis* M2 protein was partially sequenced for the design of a synthetic oligonucleotide that was employed in the screening of an *L. amazonensis* library [24].

The antigenicity and immunogenicity of *Leishmania* GP46 were analyzed. PBMCs from patients recovered of CL proliferate in response to native GP46 (but not to the recombinant protein produced in bacteria) and produce high levels of IFN-γ.In addition, the recombinant protein expressed in bacteria was recognized by the sera from VL patient and VCL dogs, indicating that some B cell epitopes are maintained in the absence of the sugar molecules moiety [95, 96].

The development of vaccines based on the rGP46 protein was evaluated in different experimental models. In the *L. major* model, protection depends on the source of GP46 antigen and the immune responses induced by the adjuvant. Whereas protection was observed with the native protein (purified from *L. major* membrane) or expressed as a recombinant protein in *L. mexicana*, vaccination with the rGP46 expressed in bacteria did not confer protection [97]. The same adjuvant, *Corynebacterium parvum*, was employed in these assays [97]. These data were taken as an indication that the native form of the antigen, including the posttranslational modifications was essential for its vaccinating potential. Similar results were obtained when the rGP46 expressed in bacteria was immunized in mice using two different adjuvants (*C. parvum* or *Quilaja* saponin) [98, 99]. The lack of protection against *L. major* infection was correlated with the induction of mixed Th1-Th2 responses. However, the immunization with an rGP46 DNA vaccine induced an exclusive Th1 response and mice were protected against an *L. major* challenge [98]. The redirection of the immune response towards a Th1 profile after GP46 DNA vaccination also had a significant therapeutic effect. In *L. major*-infected mice, vaccination with DNA encoding the GP46/M2 after parasite challenge caused reduction in the lesion size and promoted healing in both genetically resistant C3H/He and susceptible BALB/c mice [100]. In other experimental model, DNA vaccination with a recombinant expression plasmid encoding *L. amazonensis* GP46 induced *Leishmania*-specific humoral and lymphoproliferative immune responses that partially protected against a challenge with *L. mexicana* [101, 102]. Finally, immunization of susceptible BALB/c mice with an attenuated recombinant vaccinia virus expressing *L. amazonensis* GP46 induced robust protection against *L. amazonensis* challenge [103].

The *L. donovani* Kinetoplastid Membrane Protein 11 (KMP-11) is a dominant surface membrane glycoprotein associated with the LPG and is a potent T cell-stimulating factor, suggesting that it may be an important molecule for induction of cell-mediated immune responses [104, 105]. KMP-11 gene cloning was made by screening of a *L. donovani* genomic cosmid DNA library with an oligonucleotide probe designed on the basis of the partial aminoacid sequence obtained from the purified protein [25]. Afterward, *L. infantum* [106] and *L. panamensis* [107] KMP-11 coding genes were also cloned.

The use of native *L. donovani* KMP-11 as well as recombinant *L. infantum* KMP-11 as antigen in ELISA assays revealed that this protein was recognized as a B-cell antigen during human VL and canine VCL. Reactivity to native KMP-11 was found in a 58% of the serum samples from Sudanese VL patients [108]. *L. panamensis* rKMP-11 expressed in bacteria was recognized by sera from American VL (76%), MCL (60%), and CL (37%) patients [109]. A high reactivity was found against *L. infantum* rKMP-11 expressed in bacteria in the sera from VL patients infected with *L. chagasi* and lower reactivity was found in sera from individuals with subclinical *L. chagasi* infection [110]. This protein was also recognized by canine VCL sera [106]. All these results indicate that this parasite protein is a major antigenic molecule in the different clinical forms of the leishmaniasis disease. Native *L. donovani* KMP-11 is also a potent T cell stimulating antigen for $CD4^+$ murine T cells [111] and induces the up-regulation of IFN-γ mRNAs in PBMCs from dogs experimentally infected with *L. infantum* [112].

There are two studies showing that immunization with rKMP-11 conferred immunoprotection against *Leishmania* infection in different animal models. Hamsters vaccinated with a KMP-11 DNA vaccine were protected against the development of VL caused by both pentavalent antimonial sensitive and resistant virulent *L. donovani* strains [113]. The strong protection observed in this highly susceptible model correlated to the generation of Th1/Th2 mixed responses as well as $CD8^+$ T-cell activation. Furthermore, the administration of a recombinant *Toxoplasma gondii* expressing *L. major* KMP-11 induced a transient protection against CL in BALB/c mice [114].

Characterization of another *Leishmania* surface protein (HASPB1) was carried out in a search for genes preferentially expressed in infective stages of the *Leishmania* life cycle. HASPs represent a heterogeneous family of hydrophilic acylated surface proteins. The initial interest in this protein family was related to the identification of molecules associated with the change in parasite virulence. To address this question, a cDNA stage-specific library was constructed from metacyclic parasites of *L. major* and after RNA hybridization with stationary phase parasite culture RNA, a specific cDNA was found to be highly expressed in the infective form when compared to the noninfective parasites [56]. Five genes were encoded within this family and the protein products were described as surface peptide markers for infective forms of *L. major* [115, 116]. Afterward, two homologous genes were found in *L. donovani*, termed then HASPA and HASPB1. Their immunogenic properties were proved, because there was a strong recognition of infected mice sera to the recombinant HASPB1 [117]. This fact, together with the ubiquity of this protein family in

different *Leishmania* species, prompted to consider HASPB1 as a suitable candidate for immunodiagnosis and vaccine development.

A study in humans reported that more than 90% of the VL and PKDL patients under study had antibodies able to recognize rHASPB1, confirming the suitable usefulness of HASPB1 for diagnosis [118]. Moreover, rHASPB1 immunized alone induced the protection against experimental VL in the murine model, reducing both the hepatic and splenic parasite burden in vaccinated animals [119]. Immunization using rHASPB1 was assessed not only in mice, but also in a vaccine trial against canine leishmaniasis. Vaccination with rHASPB1 plus Montanide was able to induce a partial protection in 50% of the immunized dogs against infection with *L. infantum* [120].

4. Diagnostic and Vaccines Based on Intracellular Proteins

Many intracellular parasite proteins interacting with the immune system after *Leishmania* infection have been characterized. Usually, they are conserved proteins that predominantly stimulate humoral responses in VL and MCL patients, dogs suffering VCL or Th2-mediated humoral responses in experimentally infected mice. Some of these antigens are the heat shock proteins, the acidic ribosomal proteins, the nucleosome forming histones [43], a kinesine-like *Leishmania* protein [121], cysteine proteinases [122, 123], or actin and tubulin [124]. Remarkably, antibodies against some of these proteins specifically recognize the parasite antigens without cross-reactivity with the host counterparts. 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. On the other hand, although proteins that induce high humoral responses during the infectious process were not first considered as good vaccine candidates, recent works demonstrated that their vaccination may induce immunity against leishmaniasis. Table 5 summarizes some of the characterized genes encoding parasite intracellular antigens.

4.1. Ribosomal Proteins. Among the evolutionary conserved antigens of *Leishmania*, several lines of evidence suggest that ribosomal proteins are immunologically relevant molecules during infection. Some ribosomal constituents can contribute to the host immune system dysfunction through their capacity to modulate cell activities and cytokine release during infection. For example, the genetic immunization of a DNA vaccine encoding the putative 60S ribosomal protein L31 induced Th2 cytokines as well as IL-10 in mice. As a consequence of the immune response elicited, an increase in parasite burden was observed in these animals [54, 135]. Another ribosomal protein that seems to participate in the immunoregulatory processes that play a role in the balance of the Th1 and Th2 responses after infection is the *L. major* ribosomal protein S3a. This protein was purified from parasite proteins extracts using an *S*-hexylglutathione agarose affinity column [125]. The purified protein was employed for the immunization of mice and the antibodies elicited were employed to screen an *L. major* cDNA library [125]. The cDNA encoding *L. major* S3a protein was cloned into a prokaryotic expression plasmid and the purified recombinant protein was employed for the analysis of its immunogenicity [136]. rS3a was able to induce activation of B cells from normal BALB/c mice and its injection induced a non-specific polyclonal B-cell activation and inhibited T-cell proliferation [136]. Thus, it was hypothesized that the interaction of this protein released by parasite cytolysis may induce humoral responses and the down-regulation of cellular responses after parasite infection. The presence of high humoral responses against other ribosomal proteins was also documented. Thus, some members of the parasite acidic ribosomal P protein family are recognized by the sera from dogs and humans suffering VL [43, 126, 137, 138].

Three members of the *Leishmania* P protein family (namely, P0, P2a, and P2b) are constituents of the large subunit of the ribosome. They were classified according to their homology to the *Saccharomyces cerevisiae* counterparts [33, 139]. *Leishmania* P2a and P2b are anchored to the ribosomes as complexes interacting with the P0 protein, forming a protruding stalk in the ribosome major subunit. This heterocomplex plays an essential role in the elongation step of protein synthesis.

The genes encoding the *L. infantum* P2a and P2b [33] and the P0 [32] proteins were isolated and characterized after immunoscreening of a cDNA expression library with a canine VCL serum. The P2a and P2b encoding cDNAs were cloned into different prokaryotic expression vectors [138, 140] and the corresponding purified recombinant proteins were employed to analyze their antigenic and immunogenic properties. Both antigens induced a Th2 mediated humoral responses in BALB/c mice when administered without adjuvant. In addition, these responses cannot be reverted by strong Th1 inducers (DNA vaccination or coadministration of the recombinant protein with CpG ODNs) [140, 141]. According to the preponderance of the Th2 or mixed Th1/Th2 responses elicited in BALB/c mice by these inoculations, no evidence of protection was found after infection with *L. major* [140].

Both rP2 proteins have shown to be useful for diagnosis of *Leishmania* infection because substantial titres of anti-*Leishmania* P proteins antibodies are detected in VCL canine sera and in MCL and VL human patients [141, 142]. Although, the acidic ribosomal proteins were also described as prominent antigens in systemic autoimmune diseases [143], in Chagas' disease [139] and as major allergens in fungal allergies [144], the anti-Leishmania P2 humoral response is specific for the parasite antigens. This specificity was based on the location of the main antigenic determinant recognized by VCL canine sera [141]. Thus, the epitope recognized during the leishmaniasis process was found to be positioned outside the C-terminal domain, a region highly conserved among the eukaryotic P proteins [141]. Like this C-terminal region contains the antigenic determinants recognized by the sera from Chagas' disease or autoimmune patients [145], the *Leishmania* P2 proteins were engineered

Table 4: Summary of surface antigens regarding diagnosis and vaccination.

Molecule	Diagnosis	Vaccination	
	Presence of antibodies documented in	Animal model	Immunization mode
GP63	Human VL [76]	Mice CL	Liposomes GP63 [81, 82]
	Canine VCL [77]		Liposomes rGP63 [85, 86]
			DNA vaccine [90]
			Salmonella expressing rGP63 [87]
			BCG expressing rGP63 [88, 89]
			Lipopeptide [91]
			BMDCs + peptide [92]
		Mice VL	Liposomes GP63 [83, 84]
GP46	Human VL [95]	Mice CL	GP46 + *C. parvum* [97]
	Canine VCL [96]		rGP46 + *C. parvum* [97, 99]
			rGP46 + *Quilaja* [98]
			DNA vaccine [98, 101, 102]
			Vaccinia expressing GP46 [103]
KMP-11	Human VL [108–110]	Hamsters VL	DNA vaccine [113]
	Canine VCL [106]	Mice CL	*T. gondii* expressing rKMP-11 [114]
HASPB1	Human VL and PKDL [118]	Mice VL	rHASPB1 [119]
		Dogs VL	rHASPB1 + Montanide [120]

to have deletions in the C-terminal region in order to avoid cross-reactivity with sera from those patients [142].

The antigenicity and immunogenicity of the parasite P0 protein were also analyzed using recombinant versions of the protein produced in bacteria [137, 146, 147]. BALB/c mice immunization with a parasite P0-based DNA vaccine or with the rP0 protein combined with GpG ODN, showed a partial protection after challenge with *L. major* [146, 147]. P0-vaccinated mice showed an initial significant reduction in lesion size after challenge, but mice ultimately developed nonhealing lesion. The delay in the onset of cell growth was accompanied by a substantial decrease in the parasite load and was correlated to the generation of initial Th1 responses that were changed to a mixed Th1/Th2 response against the parasite rP0 when disease progressed. On the other hand, the Th1 responses induced by vaccination conferred protection against CL in C57BL/6 mice [147].

A high percentage of dogs affected with VCL contains antibodies reacting with the *L. infantum* rP0 [137]. The humoral response, as occurs for the other acidic ribosomal proteins is specifically directed against the parasite P0 protein that possesses a C-terminal region that differs from higher eukaryotes and resembles the archaebacterial acidic ribosomal protein C-terminal domain [148]. This divergent C-terminal sequence is also present in *T. cruzi* P0 and contains the antigenic determinant recognized by the sera from Chagas' disease patients. In fact, the *L. chagasi* orthologue was characterized by immunoscreening of an *L. chagasi* expression library with a pool of five *T. cruzi*-infected sera [126].

Administration of some other ribosomal constituents using immunization procedures inducing Th1 responses was related to the generation of protective responses. Thus, Melby et al. [53] showed that the genetic immunization of a mixture composed by different cDNAs encoding *L. donovani* parasite proteins including six ribosomal proteins (S14, S13, S16, S18, L8 y L28) resulted in a partial protection in BALB/c mice infected with this parasite. Also, genetic immunization with eukaryotic expression vectors containing the L22 and S19 *L. major* ribosomal protein coding genes induced a protective response in the BALB/c mice cutaneous model [54]. In addition, a cDNA clone encoding the *L. braziliensis* ribosomal protein S4 was recognized by a T-cell clone derived from a VL human donor with a positive DTH skin test, residing in an endemic area for VL [49], indicating that the recognition of some of the parasite ribosomal proteins by the host immune system is not necessarily related to disease progression (see Table 6).

4.2. Stress Proteins. The heat shock proteins (HSPs) are produced by prokaryotic and eukaryotic cells in response to a variety of physiological stresses. They are among the most highly conserved and abundant proteins found in living prokaryotic and eukaryotic organisms. As occurs in many other infectious or autoimmune diseases [149], different members of the heat shock protein family were described as antigenic after *Leishmania* infection (see Table 7).

The HSP83 protein is a member of the *Leishmania* HSP90s family located in the cytoplasm of parasite promastigotes [150]. The first parasite HSP83 coding gene was

Table 5: Genes encoding *Leishmania* intracellular antigens.

Ribosomal proteins		
Ribosomal protein S3a	(S3a)	*L. major* [125]
Ribosomal protein L31	(L31)	*L. major* [54]
Ribosomal protein S14	(S14)	*L. donovani* [53]
Ribosomal protein S13	(S13)	*L. donovani* [53]
Ribosomal protein S16	(S16)	*L. donovani* [53]
Ribosomal protein S18	(S18)	*L. donovani* [53]
Ribosomal protein L8	(L8)	*L. donovani* [53]
Ribosomal protein L28	(L28)	*L. donovani* [53]
Ribosomal protein L22	(L22)	*L. major* [54]
Ribosomal protein S19	(S19)	*L. major* [54]
Ribosomal protein S4	(S4)	*L. major* [49]
Acidic ribosomal proteins	(P2a and P2b)	*L. infantum* [33]
	(P0)	*L. infantum* [32]
		L. chagasi [126]
Stress Proteins		
Heat shock protein 20 kDa	(HSP20)	*L. amazonensis* [127]
Heat shock protein 60 kDa	(HSP60)	*L. major* [128]
Heat shock protein 70 kDa	(HSP70)	*L. donovani* [37]
		L. infantum [129]
		L. braziliensis [40]
Heat shock protein 83 kDa	(HSP83)	*L. braziliensis* [40]
		L. infantum [36]
Glucose regulated protein 94 kDa	(GRP94)	*L. infantum* [130]
Stress inducible 1	(LmSTl1)	*L. major* [41]
Histones		
Histone H1	(H1)	*L. major* [55]
Nucleosome histones	(H2A)	*L. infantum* [34]
	(H2B)	*L. infantum* [131]
	(H3)	*L. infantum* [35]
	(H4)	*L. infantum* [131]
Cysteine proteinases		
Cysteine proteinase A	(CPA)	*L. major* [132]
Cysteine proteinase B	(CPB)	*L. major* [132]
Cysteine proteinase C	(CPC)	*L. infantum* [133]
Other intracellular antigens		
Translation initiation factor 4A	(LeIF)	*L. braziliensis* [39]
Homolog receptor for activated C kinase	(LACK)	*L. major* [48]
Amastigote stage specific gene	(A2)	*L. donovani* [57]
Kinesine	(LcKin)	*L. chagasi* [134]

characterized after immunoscreening of an *L. braziliensis* expression library with a serum obtained from an MCL patient [40]. Two years later, the *L. infantum* HSP83 coding gene was rescued from a cDNA expression library using a VCL canine serum [36]. Both proteins, obtained as recombinant molecules expressed in bacteria, were employed for their serological evaluation. They were recognized as antigenic in a great percentage of patients with active CL and MCL, but anti-HSP83 antibodies were not presented in the sera from individuals with self healing CL [40, 151]. Canine VCL sera specifically recognize the *L. infantum* rHSP83 without cross-reactivity with the host HSP83 [36]. The specificity in the recognition was related with the location of the antigenic determinants in the most divergent regions of the parasite HSP83 [36]. Interestingly, rHSP83 was not recognized by the sera from chronic Chagas' disease patients [151]. For these reasons, *Leishmania* rHSP83 was considered like a potentially important diagnostic antigen. This protein also contains potent T-cell epitopes which stimulate PBMCs from MCL [40] and VL [152].

Table 6: Summary of ribosomal antigens regarding diagnosis and vaccination.

Molecule	Diagnosis	Vaccination	
	Presence of antibodies documented in	Animal model	Immunization mode
P2	Human VL and MCL [142]	Mice CL	DNA vaccine/rP2 + CpG/prime-boost [140]
	Canine VCL [138]		
P0	Canine VCL [137]	Mice CL	DNA vaccine
			rP0 + CPG [147]
S14/S13/S16/S18/L8/L28		Mice VL	DNA vaccine [53]
L22		Mice CL	DNA vaccine [54]
S19		Mice CL	DNA vaccine [54]

Table 7: Summary of stress antigens regarding diagnosis and vaccination.

Molecule	Diagnosis	Vaccination	
	Presence of antibodies documented in	Animal model	Immunization mode
HSP83	Human CL and MCL [40]		
	Canine VCL [36]		
GRP94	Human CL and MCL [154]		
	Canine VCL [154]		
HSP60	Human CL [128]		
HSP70	Human VL [37, 155, 156]	Mice CL	Prime boost [159]
	Human MCL [40, 155]		
	Human CL [40, 156]		
	Canine VCL [31, 129]		
HSP20	Canine VCL [127]	Mice CL	DNA vaccine [127]
STI1	Human CL, VL and PKDL [41]	Mice CL	rSTI1 + IL-12 [161]
	Canine VCL [59]		DNA [162]
			rSTI1 + CpG [163]

Leishmania glucose-regulated protein 94 (GRP94) is another member of the HSP90s family. This protein, located in the endoplasmic-reticulum is implicated in the LPG synthesis [153]. The antigenicity of the *L. infantum* GRP94 was evaluated after cloning of the GRP94 coding genes and expression of the rGRP94 in bacteria [130]. This protein constitutes a valuable molecule for diagnostic purposes, since 84% of sera from dogs with VCL reacted with the recombinant protein. The rGRP94 as well as synthetic peptides covering the most variable regions of the protein was also recognized by sera from MCL and VL patients [154].

There is only one report concerning the antigenicity of the *Leishmania* HSP60. A recombinant version of the *L. major* HSP60 was recognized by the sera from CL patients [128]. As occuring for the HSP83, the recognition is specific for the parasite protein since the same sera did not show reactivity with mycobacterial rHSP65 or human rHSP60 [128].

The HSP70 coding genes from several *Leishmania* species were characterized because of their recognition by leishmaniasis sera. The gene encoding *L. donovani* HSP70 was identified after screening of a cDNA library with serum from a patient with VL [37], *L. braziliensis* with an MCL serum [40] and *L. infantum* with the serum from a dog with VCL [129]. Anti-HSP70 humoral responses are highly specific against the *Leishmania* protein as occurred for the other parasite heat shock proteins. Thus, the anti-HSP70 antibodies present in the sera from infected individual did not recognize host-HSP70 [40, 129]. *Leishmania* rHSP70 could be a potential candidate for serodiagnosis since it is highly recognized by the sera from patients with VL [37, 155, 156], MCL [40, 155], CL [40, 156], and dogs with VCL [31, 129]. Although the complete protein cannot be used for specific serodiagnosis of VL because it is also recognized by the sera from Chagas' disease patients, the use of some fragments expressed as recombinant proteins or synthetic peptides covering the most divergent regions of the protein were described as valuable tools for serodiagnosis in geographical areas where mixed infections with *T. cruzi* and *Leishmania* occur [155, 156].

L. amazonensis HSP20 was recently described as an antigenic member of the heat shock protein family [127]. It was obtained as a recombinant protein expressed in bacteria and its antigenic properties were analyzed. The rHSP20 was

recognized by the sera from dogs with the VCL disease but not by the sera from human VL patients [127].

It was described that *Leishmania* HSP83 and HSP70 posses interesting adjuvant properties. Thus, the immunization of HSP83 chimeras induced Th1 responses against the fusion proteins [157]. Also, *L. infantum* HSP70 was able to induce a Th1 response against covalently linked protein, when the fusion protein was immunized in mice [158].

Some *Leishmania* heat shock recombinant products were tested as vaccines in the murine model. Thus, *L. major* HSP70 was employed to immunize mice in a prime-boost manner (DNA vaccine as a prime and a boost with the recombinant protein emulsified with Montanide) in two experimental model of murine CL (susceptible BALB/c and resistant C57BL/6 mice). A non-protective, mixed Th1/Th2 response was obtained [159]. As occur for HSP70, DNA vaccination of a HSP20 recombinant eukaryotic expression plasmid did not result in protection in murine experimental models of CL [127]. On the contrary, another member of the parasite stress protein family, the *L. major* stress-inducible 1 protein (LmSTI1) showed a protective role when it was employed as vaccine.

LmSTI1 was identified after screening of an *L. major* amastigote cDNA expression library with the sera from *L. major*-infected BALB/c mice [41]. The STI1 gene is constitutively expressed in both *L. major* promastigotes and amastigotes. However, STI1 transcript levels were upregulated in promastigotes by a shift in culture temperature from 26°C to 37°C [160]. Draining lymph nodes from *L. major* infected BALB/c mice proliferate and produce IFN-γ after in vitro restimulation with rLmSTI1 expressed in bacteria [41]. The prophylactic properties of the *L. major* STI1 were analyzed in experimental murine model of CL. Immunization of the rSTI1 adjuvated with human IL-12 [161] or as a DNA vaccine [162] induced partial protection against *L. major* infection in BALB/c mice. The degree of protection was enhanced when the rSTI1 was administered as coencapsulated in liposomes with CpG ODN [163].

The antigenicity of the *L. major* rSTI1 was also analyzed. The sera from a great percentage of patients with CL, VL, and PKDL exhibited reactivity toward rSTI1 [41]. Also, the recombinant protein was recognized by canine VCL sera [59].

4.3. Histones. Histone proteins, which are highly conserved through eukaryotic organisms, are nuclear located molecules involved in the structural formation of nucleosomes and chromatin compaction. In spite of their nuclear location, all parasite histones were described as immunodominant antigens during *Leishmania* infection [43]. The identification of an *L. infantum* cDNA encoding the H2A histone after immunoscreening with a canine VCL serum was the first report of a specific immune response against histones elicited during infection with a parasitic pathogen [34]. Afterward, a cDNA coding for the *L. infantum* H3 histone was isolated using the same methodology [35]. Both molecules, together with the other two nucleosome forming histones (H2B and H4), were obtained as recombinant proteins expressed in bacteria [131]. These recombinant molecules were proved to be antigenic in serologic assays employing canine VCL sera. The rH2A was the most frequently recognized (72%) followed by rH3 (68%), rH2B (60%), and rH4 (44%) [131]. As occurs with other *Leishmania* conserved antigens, the anti-histone humoral response elicited during canine infection is specific for the parasite antigens. Canine VCL sera did not recognize the counterpart of mammalian origin because the B cell antigenic determinants were located in the most divergent regions of the parasite histones: the amino-terminal ends of the four core histones and also the C-terminal region of the H2A [131, 164, 165]. In addition, it was found that 58% of patients with American CL have antibodies reacting with the *L. peruviana* H2B [166], that all the sera from patients with VL caused by *L. chagasi* reacted against the *L. infantum* H2A [110] and that high percentages of VL patients from the Mediterranean area had anti-*Leishmania* H2A- and H2B-specific antibodies [95]. All these data indicate that histones can be taken into account in the development of serodiagnosis systems based on recombinant parasite antigens.

The T cell immunogenicity of parasite histones was also demonstrated, since *Leishmania* rH2B protein was able to induce strong in vitro proliferation and IFN-γ production in PBMCs obtained from patients with CL and from a T-cell clone derived from a VL immune donor [49]. Also, a predominant IFN-γ production was observed when PBMCs from CL patients were stimulated with *L. infantum* rH2A and rH3 histones [167].

The prophylactic value of the *Leishmania* histones was evaluated in different experimental models. It was firstly described that the immunization of a mixture of expression eukaryotic plasmids encoding *L. donovani* H2B, H3, and H4 was able to induce partial protection against infection with *L. donovani* in mice [53]. In addition, the co-administration of a mixture of the four *L. infantum* nucleosomal histones as a DNA vaccine [168] protected BALB/c mice against a virulent challenge with *L. major*. The observed protection was related with the generation of histone-specific Th1 response in which both $CD8^+$- and $CD4^+$-dependent production of IFN-γ was detected [168]. Also, the adoptive transfer of BMDCs pulsed with the four parasite recombinant histones plus CpG ODN induced protection in CL [169, 170] and VL [171] murine models. Finally, it was documented that the immunization of a recombinant protein containing the divergent amino-terminal region of the *L. major* H2B was also protective in the murine model of CL when adjuvated with CpG ODN [172].

Histone H1 was related with parasite infectivity because it is overexpressed early during infection. *L. major* H1 histone coding gene was isolated from a parasite cDNA expression library constructed with mRNA from macrophages recently infected with *L. major* [55]. Generation of *L. major* transfectants overexpressing histone H1 causes a delay in the cell-cycle progression [173] that reduces infectivity in vitro [174] and in vivo [173]. Interestingly, a vaccine composed of *L. major* H1 partially purified or obtained as a recombinant protein had protective capacity against experimental murine CL [175]. Furthermore, it was observed that combination of the rH1 protein plus Montanide (an adjuvant employed

Table 8: Summary of histone antigens regarding diagnosis and vaccination.

	Diagnosis	Vaccination	
Molecule	Presence of antibodies documented in	Animal model	Immunization mode
H2s/H3/H4	Canine VCL [131, 164]	Mice CL	DNA vaccine [168]
		Mice CL	BMDCs + CpG [169]
		Mice VL	BMDCs + CpG [171]
H2B	Human VL [95]	Mice CL	rH2B-Nt + CpG [172]
	Human CL [166]		
H2A	Human VL [95]		
H2A/H3/H4		Mice VL	DNA vaccine [53]
H1		Mice CL	rH1 [175]
		Monkey CL	rH1 + Montanide [176]

for human vaccination) developed protection against CL in outbred vervet monkeys [176] (see Table 8).

4.4. Cysteine Proteinases. Cysteine proteinases (CPs) are key molecules for *Leishmania* virulence. Because of their critical contribution to the ability of the parasites to infect and proliferate in mammals, cysteine proteinases of *Leishmania* were viewed as promising drug targets for many years [177]. These enzymes seem to be predominantly expressed and active in amastigotes and to a lesser extent in metacyclic promastigotes [178, 179]. Three classes of cysteine proteinase genes (types I–III) were identified in *Leishmania*: CPB genes (type I), CPA (type II), and CPC (type III). Although the exact role of cysteine proteinases in *Leishmania* pathogenesis remains unclear, it was demonstrated that *Leishmania* cannot grow within macrophages in the presence of cysteine proteinase inhibitors [180]. Also, the gene product of *cpb 2.8* of *L. mexicana* is a potent inducer of a Th2 response in BALB/c mice [122]. In addition, it was demonstrated that the CPB of this parasite suppresses the antileishmanial Th1 immune response of C3H and C57BL/6 mice [181]. Moreover, deletion of CPs genes diminishes pathogenicity of *L. mexicana* in hamsters and *cpa/cpb*-deficient *L. mexicana* grew more slowly as promastigotes presenting lower infectivity and growth in human mononuclear phagocyte host cells [182]. All these data provide evidence of the importance of these molecules in the survival of both promastigote and amastigote forms of *Leishmania* parasites.

The CPA and CPB genes from different *Leishmania* species were isolated and the antigenicity and immunogenicity of the corresponding recombinant proteins were analyzed. *L. major* CPA and CPB genes were obtained using specific primers designed from the sequence of *L. mexicana* CPs and genomic DNA from *L. major* promastigotes [132]. PCR products corresponding to *L. major* CPB and CPA genes were amplified, cloned, and subsequently expressed as recombinant proteins in bacteria. The same primers were employed to obtain *L. infantum* CPs genes [123]. The *L. chagasi* CPB and the *L. infantum* CPC [133] proteins were also obtained as recombinant proteins expressed in bacteria.

Sera from active or recovered cases of human CL patients showed a high reactivity against *L. major* rCPs [132]. Similar and intensive recognition of *L. infantum* rCPs (with a higher recognition toward rCPB than rCPA) was observed in active cases of human VL [123]. These proteins were also recognized by the sera from dogs affected with VCL [123]. The *L. chagasi* CPB (that presents a high degree of conservation with the *L. infantum* CPB) was described as a tool suitable for the diagnosis of human VL since it was recognized by the sera from VL patients (80% of sensitivity) without cross-reactivity with the sera from other diseases, including Chagas' disease and tuberculosis (96% of specificity) [183]. Also, *L. infantum* rCPC was recognized by the sera from active and cured VL patients [133].

The immunogenicity of the rCPs was also evaluated. *L. major* rCPA and rCPB induced Th1 responses in patients with localized CL due to *L. guyanensis* (neither IL-4 nor IL-13 and low levels of IL-10 were detected) [184]. In addition, it was shown that the PBMCs obtained from individuals recovered from CL, produced IFN-γ after in vitro stimulation with a chimerical protein composed by both *L. major* CPs [185]. Also, and for canine VCL, asymptomatic dogs exhibited specific lymphocyte proliferation to *L. infantum* rCPs in contrast to the symptomatic cases [123]. Finally, it was described that *L. chagasi* rCPB induces the in vitro proliferation and secretion of IFN-γ in PBMCs obtained from *L. chagasi* infected asymptomatic humans and dogs [186]. Interestingly, symptomatic subjects produced lower levels of IFN-γ, and also IL-4 and IL-10 in response to the stimulation with rCPB [186].

Several studies determined the efficacy of vaccines based on CPs against CL in mice. DNA vaccines encoding *L. major* CPs were tested, administrated intramuscularly in BALB/c mice, either separately or as a cocktail [187]. It was only when the *cpa* and *cpb* genes were coinjected that long-lasting protection against parasite challenge was achieved. Analysis of the immune response showed that protected animals developed a specific Th1 immune response, which was associated with an increase of IFN-γ production. A similar protection was also observed when animals were primed with *cpa/cpb* DNA followed by recombinant CPA/CPB boost [187]. The recombinant *L. major* rCPB or rCPA inoculated together with poloxamer as adjuvant was tested in the same infection model [188]. Vaccination with rCPB, but not

Table 9: Summary of cysteine proteinases antigens regarding diagnosis and vaccination.

Molecule	Diagnosis	Vaccination	
	Presence of antibodies documented in	Animal model	Immunization mode
CPA/CPB	Human CL [132, 183]	Mice CL	DNA vaccine [187]
	Human VL [123]		Prime-boost [187]
	Canine VCL [123]		rCPs + poloxamer [185, 188]
		Mice VL	Prime-boost [189]
		Dogs VL	Prime-boost [190]
			rCPs + IL-12 [191]
			rCPs + Quil A [191]
CPC	Human VL [133]	Mice VL	Prime boost [133]

rCPA, allowed BALB/c mice to mount a partial protective response, with a delay in the clinical outcome. This partial protective effect was abrogated if a CD8 depleting antibody was given intravenously to rCPB-immunized mice, at the time of parasite challenge. In fact, only one immunological parameter, namely, the higher frequency of IFN-γ producing $CD8^+$ T lymphocyte after challenge in the draining lymph nodes, correlated to the partial protection achieved by the injection of rCPB plus poloxamer. Therefore, to reduce the production costs, the *cpa* and *cpb* genes were fused in tandem together to give rise to a single hybrid protein [185]. The protective potential of the CPA/B hybrid protein plus poloxamer against *L. major* infection was then assessed in BALB/c mice, showing a delay in the expansion of lesions size compared to control groups. A predominant Th1 immune response characterized by in vitro IFN-γ specific production and predominant IgG2a subclass antibodies was observed [185].

CPs-based vaccines were also tested against experimental VL. The efficacy of vaccination with a cocktail of DNA encoding CPs followed by a boost with rCPA/rCPB adjuvated with CpG ODN and Montanide was tested in the experimental murine model of *L. infantum* infection in BALB/c mice [189]. The immune response elicited by this vaccine was of the Th1-type (a higher ratio of IgG2a/IgG1-specific antibodies, beside a higher ratio of IFN-γ/IL-5 induced upon restimulation with rCPA and rCPB in vaccinated group compared to control groups). In the liver, the parasite burden peaked with some delay in vaccinated mice and a complete clear of the infection in both liver and spleen was observed [189]. The same model of infection was employed to test a CPC-based vaccine [133]. Mice immunized with a CPC DNA vaccine and boosted with the rCPC protein plus CpG ODN and Montanide showed lower parasite burden in the spleen and in the liver than controls immunized with the adjuvants alone after *L. infantum* infection [133].

Vaccines based on *Leishmania* CPs were also tested in dogs. A heterologous prime-boost regime was assessed, consisting of DNA/recombinant *L. infantum* CPs (in combination with CpG ODN and Montanide) [190]. In contrast to control groups, dogs vaccinated by prime/boost remained free of *L. infantum* parasites in their bone marrow. Protection correlated the higher levels of total IgG and IgG2 (but not IgG1) to rCPA and rCPB. PBMCs from vaccinated dogs showed higher level of proliferation than controls when stimulated with total parasite antigens or rCPs [190]. Proliferation correlated to the presence of higher IFN-γ mRNA and less IL-10 mRNA levels [190]. Notwithstanding, other study reported that administration of *L. infantum* rCPA and rCPB adjuvated with recombinant canine IL-12 alone or in combination with Quil A was not effective to vaccinate dogs against an intravenous challenge with *L. infantum* [191] (see Table 9).

4.5. Other Intracellular Antigens. The homologous of the higher eukaryotic initiation factor 4A (eIF4A) in *Leishmania* (LeIF) was described as an immunostimulatory antigen. It was identified by screening of an *L. braziliensis* genomic expression library with a serum from a patient suffering MCL [39]. Subsequently, it was shown that the recombinant protein stimulates the production of Th1 cytokines and the proliferation of the T cells from this patient [39]. This study revealed several evidences indicating that LeIF protein promotes preferentially Th1-type responses after infection. Thus, rLeIF induced higher proliferative response in patients with MCL and self-healing CL disease than those with cutaneous lesions. Whereas the parasite lysate stimulated patient PBMCs to produce a mixed Th1/Th2-type cytokine profile, rLeIF stimulated the production of IFN-γ, IL-2, and TNF-α but not IL-4 or IL-10, besides down-regulating IL-10 mRNA expression. rLeIF also stimulated the production of IL-12 in culture and the IFN-γ production was IL-12, dependent [39]. Further, it was observed that rLeIF protein stimulates human monocytic antigen-presenting cells to produce IL-12, concomitantly with an upregulation of costimulatory and intercellular adhesion molecules [192]. The immunogenicity of the parasite LeIF was also analyzed in experimental CL infected mice. The in vitro stimulation of draining lymph node cells from *L. major*-infected BALB/c mice with rLeIF preferentially secreted IFN-γ (no detectable IL-4 production was found) [193]. In addition, rLeIF downregulated *Leishmania* Ag-specific IL-4 production by lymph node cells from infected BALB/c mice. Subsequently, BALB/c mice immunized with rLeIF were partially protected

Table 10: Summary of other intracellular antigens regarding diagnosis and vaccination.

	Diagnosis	Vaccination	
Molecule	Presence of antibodies documented in	Animal model	Immunization mode
eIF4a		Mice CL	rLeIF [193]
LACK		Mice CL	rLACK + IL-12 [48, 203]
			DNA vaccine [102, 196, 198, 205]
			Listeria expressing rLACK [199]
			Vaccinia expressing LACK [200–202]
		Mice VL	Prime-boost [210]
			DNA vaccine [206, 207]
		Dogs VL	Prime-boost [208, 209]
A2	Human VL [213, 214]	Mice VL	DNA vaccine [215]
	Canine VL [214]		rA2 + *P. acnes* [216]
		Dogs VL	rA2 + saponine [217]
K39	Human VL [95, 121, 134, 218–222]		
	Canine VL [223–226]		
Kinesine motor domain	Human VL [227]		

against *L. major* infection. Finally it was found that rLeIF stimulated fresh spleen cells from naive SCID mice to secrete IFN-γ by IL-12/IL-18-dependent mechanisms [193]. Since SCID mice lack T and B lymphocytes but have a normal innate immune system (normal reticuloendothelial system and NK cells), it was suggested that LeiF might be considered a microbial pattern recognition molecule. In addition, rLeiF induce IFN-γ production by NK cells [194]. The putative receptor of LeiF is unknown, yet Toll-like receptor 4 (TLR4) was excluded, since LeiF was able to stimulate splenocytes from mice defective for this receptor (C3H/HeJ) [194]. Thus, all these data suggested that LeIF might be used as a Th1-type adjuvant as well as a therapeutic and prophylactic vaccine antigen for leishmaniasis (see Table 10).

LACK protein (the leishmanial homolog of mammalian receptor for activated C kinase) is probably the best characterized antigen of *L. major*. It was firstly described using a protective $CD4^+$ T cell clone (Th1) from immunized BALB/c mice [47] to screen an epitope-tagged expression library. A conserved 36-kilodalton member of the tryptophan-aspartic acid repeat family of proteins was identified that is expressed in both stages of the parasite life cycle [48]. Interestingly, it was shown that this parasite protein was implicated in the induction of the early IL-4 response against *L. major* occurring in susceptible BALB/c [195], since this $CD4^+$ T-mediated response occurs mainly by a restricted population of $CD4^+$ T cells that expressed the Vα8Vβ4 TCR chains that are specific for LACK [195]. However, the coadministration of rLACK with Th1 adjuvants in BALB/c mice was able to redirects the naturally induced Th2 responses after *L. major* infection. Thus, rLACK protein administered with interleukin-12 [48] or an LACK-based DNA vaccine [196] protected BALB/c mice against *L. major* infection. The essential role of LACK in the aberrant Th2 response of susceptible mice against *L. major* was further confirmed by data showing that BALB/c rendered tolerant to LACK, as a result of transgenic expression of this molecule in the thymus, were resistant to infection with *L. major* and develop a Th1 response after infection [197]. In summary, all these data support the outstanding protection induced by several vaccination protocols based on LACK antigen against *L. major* infection in BALB/c mice [198–202] a CL model that depends critically on a Th1/Th2 balance. However, vaccines based on LACK were not so efficient to induce protection against other *Leishmania* species that are not so strictly dependent on this Th1/Th2 balance, namely, *L. mexicana* [102, 203], *L. amazonensis* [203–205], *L. donovani* [206], or *L. chagasi* [207]. Notwithstanding, it was demonstrated that dogs experimentally infected with *L. infantum* were protected against VL following an heterologous prime-boost vaccination regime with a DNA vaccine encoding LACK and recombinant vaccinia virus (rVACV) expressing LACK [208], or its corresponding nonreplicative modified vaccinia (MVA-LACK) [209]. Similarly, the same prime-boost regime protects BALB/c mice against an intradermal infection with this parasite [210] (see Table 10).

The *L. donovani* A2 multigene family encodes for a group of proteins that are composed predominantly of multiple copies of a 10 aminoacid repeated sequence ranging in molecular weight from 45 to 110 kDa, depending on the number of repeats within the protein [211]. The A2 family was characterized looking for molecules specific of the amastigote stage. These genes were isolated after the screening of an amastigote cDNA library with life cycle stage-specific DNA probes [57]. *L. chagasi* A2 gene was also identified after a double screening of an amastigote cDNA library using in a first step a pool of sera from Brazilian VL patients and a second step T cells obtained from immunne mice [212]. Since the *Leishmania* A2 antigen showed immunogenic properties, it was obtained as a recombinant protein expressed in bacteria and tested for a possible role in diagnosis and vaccination. A study using the

L. donovani rA2 protein, performed with kala-azar patients from an endemic region, showed that it was possible to detect by ELISA anti-A2 antibodies in 82% of the VL patients in Sudan and 60% in India, meanwhile using antibody immunoprecipitation the detection rate increased up to 92% [213]. A similar study was done in VCL dogs and Brazilian patients of VL. Anti-A2 antibodies were found in the 87% of dogs and 77% of the symptomatic patients, suggesting that the rA2 protein would be of particular interest for serodiagnosis [214].

Immunization of both *L. donovani* A2 DNA vaccines [215] or rA2 protein combined with *Propionibacterium acnes* [216] was proved to induce a significant protection against VL caused by *L. donovani* in experimentally infected mice. Protection correlated to the generation of a Th1/Th2 mixed response and with the A2-specific splenocyte proliferation and production of IFN-γ, and also with the generation of anti-A2 antibodies that induce a complement-mediated reduction of the viability of amastigotes that results in a reduction of macrophage infection [216]. Immunization of the *L. donovani* rA2 protein formulated with saponine, induced protection against *L. chagasi* experimental infection in dogs [217] (see Table 10).

An *L. chagasi* antigenic protein of great value for the generation of canine and human VL diagnosis test was named rK39. This recombinant protein contains an extensive repetitive domain located in the C-terminal region of the *L. chagasi* kinesine (LcKin) and was isolated after screening of an *L. chagasi* genomic expression library with a serum of a VL patient infected with *L. donovani* [134]. LcKin belongs to a conserved microtubule-based motor protein superfamily and possesses a conserved sequence in the motor domain but little sequence similarity outside of the domain, including the repetitive aminoacid sequence encoded by the K39 clone [134]. The rK39 protein was employed for human diagnosis of VL caused by the related *L. chagasi* and *L. infantum* species as well as VL caused by *L. donovani* [95, 121, 134, 218–222] and for diagnosis of canine VCL [223–226]. Tests based on this antigen have high sensitivity (around 100%) and large specificity, since anti-K39 antibodies are virtually absent in the sera from patients with CL, MCL, or Chagas' disease [134]. Since sera from early infected of self-healing subjects were nonreactive with rK39 and the antibody titres to this antigen directly correlated to active disease, it can be employed as marker for disease progression in VL [121]. In addition rK39 ELISA has a high predictive value for detecting VL in immunocompromised persons, like those with AIDS [219]. A kit (InBios, USA) using this antigen is now commercially available in the form of antigen-impregnated nitrocellulose paper strips adapted for use under field conditions.

Remarkably, and as also occurs for other intracellular antigens, the aminoacids repeats of the parasite kinesin contaied in the rK39 protein possessed epitopes that cause proliferation and IFN-γ production in T cells isolated from immune mice [212].

The antigenicity and immunogenicity of the conserved motor domain region located in the N-terminal region of the *L. chagasi* kinesine, were also analyzed. For that purpose a recombinant protein containing the N-terminal region of *L. donovani* kinesine was expressed in bacteria [227]. This recombinant protein induced the proliferation of PBMCs and was recognized by the sera from VL cured patients [227] (see Table 10).

5. Vaccines or Diagnosis Based on Mixtures of Nonrelated Antigens or Poly-Protein Quimeric Molecules

Humans or dogs naturally exposed to *Leishmania* mounted both humoral and cellular responses to some of the above antigens. The fact that none of the antigen elicited responses in all exposed individuals underscores the fact that a vaccine formulation and optimized diagnostic test based on recombinant proteins may require a mixture of parasite antigens. Then, efficient diagnosis as well as the development of vaccines based on recombinant proteins will require the combination of nonrelated antigenic molecules.

In this sense, Dumonteil et al. tested DNA vaccines encoding *L. mexicana* GP63, CPB, and LACK, as well as *L. amazonensis* GP46 in the BALB/c model of *L. mexicana* infection [101]. Although each one of the four DNA vaccines induced *Leishmania*-specific humoral and lympho-proliferative immune responses, only mice immunized with GP46, GP63, and CPB were partially protected against challenge and, moreover, the immunization of mice with a mixture of these three plasmids further increased protection. A DNA-vaccine based on a mixture of TSA, STI1, and LACK genes was evaluated in the cutaneous model that more accurately reproduces the clinical-pathological findings associated with human disease, consisting in the intradermal inoculation of a low number of *L. major* parasites into the ear of resistant C57BL/6 mice [228]. Vaccination with a mixture of these genes protected C57BL/6 and BALB/c mice even when challenged 12 weeks after the immunization (no pathology and a 1000-fold reduction in dermal parasite loads) [229]. Concomitantly, administration of the rSTI1 and rTSA proteins adjuvated with alum and human IL-12 induced a remarkable protection in *Rhesus* monkeys, being a promising candidate subunit vaccine against human leishmaniasis [161]. The same combination of STI1 and TSA antigens was tested as a DNA vaccine in BALB/c mice challenged with *L. major* in the footpad. TSA-DNA vaccine conferred a substantial protection greater than the partial protection induced by STI1-DNA. Interestingly, this different degree of protection correlated to the activation of $CD8^+$ T cell responses, since cytotoxic-T-lymphocyte activity was generated after immunization with TSA DNA but not STI1 DNA [162]. The immunogenicity of TSA, LeIF, and STI1 recombinant antigens combined with MPL-SE or AdjuPrime was also analyzed in dogs as potential vaccine candidates for VCL [230]. When dogs immunized with the recombinant antigens plus MPL-SE were experimentally exposed to low numbers of culture forms of *L. chagasi* promastigotes a clear boost in the immune response was observed. Moreover, immunoglobulins were predominantly of the IgG2 isotype, whereas animals primed with the recombinant antigens formulated in AdjuPrime as well as animals vaccinated with

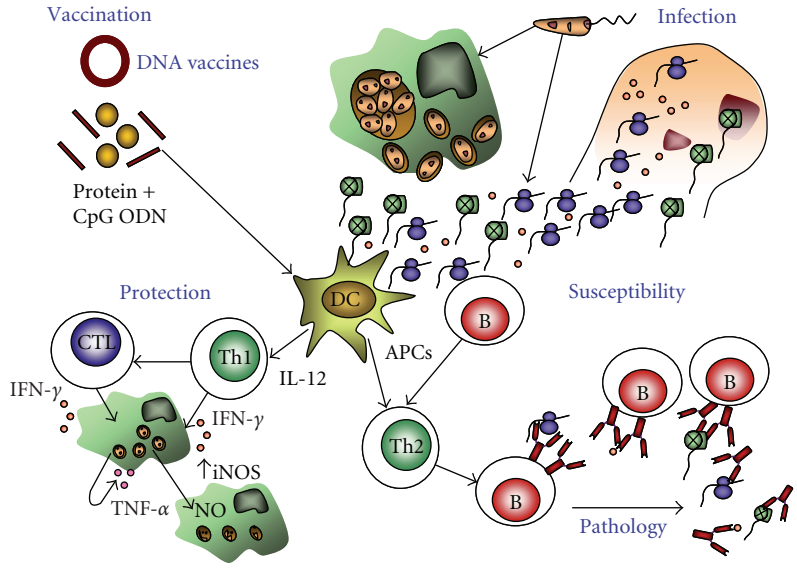

Figure 3: A model of pathology and protective capacity for the *Leishmania* intracellular antigens. Intracellular parasite antigens are presented to the immune system during the natural course of infection and stimulate antigen-specific Th2-mediated humoral responses. The induction of a Th1 immune response to them is an interesting approach to the development of *Leishmania* vaccines.

crude antigen preparation responded with mixed IgG1/IgG2 isotypes [230].

In order to facilitate the transfer from laboratory to the field, the production of polyproteins containing several parasite antigens will reduce the cost of the recombinant products. For this reason, a multisubunit recombinant leishmanial vaccine was developed based on both TSA and STI1 antigens, besides LeiF. A single recombinant polyprotein was constructed including the sequences of all three open reading frames genetically linked in tandem, comprising an unique open reading frame coding for a 111 kDa polypeptide (Leish111f) [231]. Leish111f adjuvated with MPLE-SE protected mice against CL caused by *L. major* in BALB/c mice [231] and *L. amazonensis* in C57BL/6 mice [232]. The immunogenicity and protection induced by Leish-111f formulated with monophosphoryl lipid A in a stable emulsion (Leish-111f+MPL-SE) was further tested against VL caused by *L. infantum*. Experimental infection of immunized mice and hamsters demonstrated that Leish-111f + MPL-SE induced significant protection against *L. infantum* infection (reductions in parasite loads of 99.6%, greater than that reported for other vaccine candidates in these animal models of VL) [233]. In summary, all these data suggest that this vaccine represents a good candidate for use against several *Leishmania* species. In fact, the Leish-111f + MPL-SE product is the first defined vaccine for leishmaniasis in human clinical trials and has completed phase 1 and 2 (safety and immunogenicity testing in normal, healthy human subjects) [9].

Other parasite multiepitope proteins were also constructed. Boarino et al. assayed a recombinant chimeric antigen containing the 39 aminoacid unit of *L. chagasi* K39 fused with other two *L. chagasi* antigens (K9 and K26) for the diagnosis of human VL and canine VCL [234]. Also, a chimeric protein containing the antigenic determinant of the *L. infantum* histone H2A and the acidic ribosomal protein P2a, P2b and P0 (namely, protein-Q) was constructed for the specific diagnosis of canine VCL with a sensitivity ranging from 79% to 93% and specificity ranging for 96% to 100% dependent of the negative control sera employed [235]. The ability of this multiantigenic protein to induce protection was tested in a murine model of VL [236]. Administration of this protein with CpG ODN induces a mixed Th1/Th2 response that results in a significant protection after *L. infantum* challenge in BALB/c mice [236]. Administration of the protein-Q mixed with live BCG conferred protection to dogs experimentally infected with *L. infantum* [237].

6. Concluding Remarks

In the detection of leishmaniasis cases, serodiagnostic methods are of great importance prior to attempts to parasite detection. Different parasite antigenic proteins were isolated and expressed as recombinant proteins but the potential diagnostic usefulness of these recombinant proteins for human and canine leishmaniasis deserves further research. This includes the analysis of all the available recombinant proteins against the sera from patients or dogs living in endemic areas and the evaluation of their reactivity with the sera from other cross-reacting diseases. The identification of new parasite specific antigens or specific antigenic determinants in the already characterized proteins will allow the development of more sensitive and specific tests.

Although a common feature of most of the *Leishmania* proteins described above is their frequency of recognition by sera from leishmaniasis patients, many of them were also considered of interest for the development of vaccines. Surface, secreted and excreted antigens were tested for their

prophylactic potential because they are the first parasite factors that interact with the host immune system and are usually implicated in the establishment of the infection. Moreover, many intracellular proteins referred as "pathoantigens" because an inadequate humoral response against them is thought to result in pathology [42, 238, 239] are good candidates for vaccination if their Th2 mediated responses are redirected by the use of Th1 adjuvants (Figure 3).

Generation of vaccines against such a complex parasite as *Leishmania* would be optimized by incorporating different target antigens in the vaccine formulation, taking advantage of these antigens that induce the required immunity (mainly $CD4^+$ and $CD8^+$ IFN-γ-mediated responses) and redirecting towards a Th1 bias the pathoantigenic-driven immune responses that result in pathology (IL-4 Th2-driven and IL-10 deactivating responses).

Acknowledgments

This study was supported by the Ministerio de Ciencia e Innovación and the Instituto de Salud Carlos III within the Network of Tropical Diseases Research (RICET RD06/0021/0008). Grants from Ministerio de Ciencia e Innovación (FIS/PI080101), from Ministerio de Educación y Ciencia (PHB2006-0006-PC), from AECID (A/7692/07 and A/016407/08/08), and a grant from CYTED (207RT0308) are acknowledged. An institutional grant from Fundación Ramón Areces is also acknowledged.

References

[1] B. L. Herwaldt, "Leishmaniasis," *The Lancet*, vol. 354, no. 9185, pp. 1191–1199, 1999.

[2] M. Gramiccia and L. Gradoni, "The current status of zoonotic leishmaniases and approaches to disease control," *International Journal for Parasitology*, vol. 35, no. 11-12, pp. 1169–1180, 2005.

[3] G. M. Santos-Gomes, R. Rosa, C. Leandro, S. Cortes, P. Romão, and H. Silveira, "Cytokine expression during the outcome of canine experimental infection by *Leishmania infantum*," *Veterinary Immunology and Immunopathology*, vol. 88, no. 1-2, pp. 21–30, 2002.

[4] L. Gradoni, "An update on antileishmanial vaccine candidates and prospects for a canine *Leishmania* vaccine," *Veterinary Parasitology*, vol. 100, no. 1-2, pp. 87–103, 2001.

[5] D. McMahon-Pratt and J. Alexander, "Does the *Leishmania major* paradigm of pathogenesis and protection hold for New World cutaneous leishmaniases or the visceral disease?" *Immunological Reviews*, vol. 201, no. 1, pp. 206–224, 2004.

[6] H. D. F. H. Schallig and L. Oskam, "Molecular biological applications in the diagnosis and control of leishmaniasis and parasite identification," *Tropical Medicine and International Health*, vol. 7, no. 8, pp. 641–651, 2002.

[7] K. Kar, "Serodiagnosis of leishmaniasis," *Critical Reviews in Microbiology*, vol. 21, no. 2, pp. 123–152, 1995.

[8] J. Kubar and K. Fragaki, "Recombinant DNA-derived *Leishmania* proteins: from the laboratory to the field," *The Lancet Infectious Diseases*, vol. 5, no. 2, pp. 107–114, 2005.

[9] R. N. Coler and S. G. Reed, "Second-generation vaccines against leishmaniasis," *Trends in Parasitology*, vol. 21, no. 5, pp. 244–249, 2005.

[10] E. Handman, "Leishmaniasis: current status of vaccine development," *Clinical Microbiology Reviews*, vol. 14, no. 2, pp. 229–243, 2001.

[11] F. Modabber, "Vaccines against leishmaniasis," *Annals of Tropical Medicine and Parasitology*, vol. 89, supplement 1, pp. 83–88, 1995.

[12] C. L. Greenblatt, "The present and future of vaccination for cutaneous leishmaniasis," *Progress in Clinical and Biological Research*, vol. 47, pp. 259–285, 1980.

[13] O. I. Kellina, "Problem and current lines in investigations on the epidemiology of leishmaniasis and its control in the U.S.S.R," *Bulletin de la Societe de Pathologie Exotique et de ses Filiales*, vol. 74, no. 3, pp. 306–318, 1981.

[14] C. B. Palatnik, R. Borojevic, J. O. Previato, and L. Mendonça-Previato, "Inhibition of *Leishmania donovani* promastigote internalization into murine macrophages by chemically defined parasite glycoconjugate ligands," *Infection and Immunity*, vol. 57, no. 3, pp. 754–763, 1989.

[15] C. B. Palatnik-de-Sousa, E. M. Gomes, E. Paraguai-de-Souza, M. Palatnik, K. Luz, and R. Borojevic, "*Leishmania donovani*: titration of antibodies to the fucose-mannose ligand as an aid in diagnosis and prognosis of visceral leishmaniasis," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 89, no. 4, pp. 390–393, 1995.

[16] G. P. Borja-Cabrera, V. O. Da Silva, R. T. Da Costa, et al., "The fucose-mannose ligand-ELISA in the diagnosis and prognosis of canine visceral leishmaniasis in Brazil," *The American Journal of Tropical Medicine and Hygiene*, vol. 61, no. 2, pp. 296–301, 1999.

[17] L. E. Parra, G. P. Borja-Cabrera, F. N. Santos, L. O. P. Souza, C. B. Palatnik-de-Sousa, and I. Menz, "Safety trial using the Leishmune® vaccine against canine visceral leishmaniasis in Brazil," *Vaccine*, vol. 25, no. 12, pp. 2180–2186, 2007.

[18] C. B. Palatnik-de-Sousa, H. S. Dutra, and R. Borojevic, "*Leishmania donovani* surface glycoconjugate GP36 is the major immunogen component of the Fucose-Mannose Ligand (FML)," *Acta Tropica*, vol. 53, no. 1, pp. 59–72, 1993.

[19] J.-L. Lemesre, P. Holzmuller, M. Cavaleyra, R. B. Gonçalves, G. Hottin, and G. Papierok, "Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of *Leishmania infantum* promastigotes," *Vaccine*, vol. 23, no. 22, pp. 2825–2840, 2005.

[20] J.-L. Lemesre, P. Holzmuller, R. B. Gonçalves, et al., "Long-lasting protection against canine visceral leishmaniasis using the *Li*ESAp-MDP vaccine in endemic areas of France: double-blind randomised efficacy field trial," *Vaccine*, vol. 25, no. 21, pp. 4223–4234, 2007.

[21] R. Rosa, O. R. Rodrigues, C. Marques, and G. M. Santos-Gomes, "*Leishmania infantum*: soluble proteins released by the parasite exert differential effects on host immune response," *Experimental Parasitology*, vol. 109, no. 2, pp. 106–114, 2005.

[22] R. Rosa, C. Marques, O. R. Rodrigues, and G. M. Santos-Gomes, "Immunization with *Leishmania infantum* released proteins confers partial protection against parasite infection with a predominant Th1 specific immune response," *Vaccine*, vol. 25, no. 23, pp. 4525–4532, 2007.

[23] L. L. Button and W. R. McMaster, "Molecular cloning of the major surface antigen of *Leishmania*," *Journal of Experimental Medicine*, vol. 167, no. 2, pp. 724–729, 1988.

[24] K. L. Lohman, P. J. Langer, and D. McMahon-Pratt, "Molecular cloning and characterization of the immunologically protective surface glycoprotein GP46/M-2 of *Leishmania amazonensis*," *Proceedings of the National Academy of Sciences*

of the United States of America, vol. 87, no. 21, pp. 8393–8397, 1990.

[25] A. Jardim, S. Hanson, B. Ullman, W. D. McCubbin, C. M. Kay, and R. W. Olafson, "Cloning and structure-function analysis of the *Leishmania donovani* kinetoplastid membrane protein-11," *Biochemical Journal*, vol. 305, part 1, pp. 315–320, 1995.

[26] C. E. Clayton, "Life without transcriptional control? From fly to man and back again," *The EMBO Journal*, vol. 21, no. 8, pp. 1881–1888, 2002.

[27] J. E. Donelson, M. J. Gardner, and N. M. El-Sayed, "More surprises from *Kinetoplastida*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 6, pp. 2579–2581, 1999.

[28] J. R. Webb, A. Campos-Neto, P. J. Ovendale, et al., "Human and murine immune responses to a novel *Leishmania major* recombinant protein encoded by members of a multicopy gene family," *Infection and Immunity*, vol. 66, no. 7, pp. 3279–3289, 1998.

[29] B. Yahiaoui, M. Loyens, A. Taibi, R. Schöneck, J. F. Dubremetz, and M. A. Ouaissi, "Characterization of a *Leishmania* antigen associated with cytoplasmic vesicles resembling endosomal-like structure," *Parasitology*, vol. 107, part 5, pp. 497–507, 1993.

[30] P. J. Murray, T. W. Spithill, and E. Handman, "The PSA-2 glycoprotein complex of *Leishmania major* is a glycosylphosphatidylinositol-linked promastigote surface antigen," *The Journal of Immunology*, vol. 143, no. 12, pp. 4221–4226, 1989.

[31] L. Quijada, J. M. Requena, M. Soto, et al., "Mapping of the linear antigenic determinants of the *Leishmania infantum* Hsp70 recognized by leishmaniasis sera," *Immunology Letters*, vol. 52, no. 2-3, pp. 73–79, 1996.

[32] M. Soto, J. M. Requena, and C. Alonso, "Isolation, characterization and analysis of the expression of the *Leishmania* ribosomal PO protein genes," *Molecular and Biochemical Parasitology*, vol. 61, no. 2, pp. 265–274, 1993.

[33] M. Soto, J. M. Requena, M. Garcia, L. C. Gomez, I. Navarrete, and C. Alonso, "Genomic organization and expression of two independent gene arrays coding for two antigenic acidic ribosomal proteins of *Leishmania*," *The Journal of Biological Chemistry*, vol. 268, no. 29, pp. 21835–21843, 1993.

[34] M. Soto, J. M. Requena, L. C. Gomez, I. Navarrete, and C. Alonso, "Molecular characterization of a *Leishmania donovani infantum* antigen identified as histone H2A," *European Journal of Biochemistry*, vol. 205, no. 1, pp. 211–216, 1992.

[35] M. Soto, J. M. Requena, G. Morales, and C. Alonso, "The *Leishmania infantum* histone H3 possesses an extremely divergent N-terminal domain," *Biochimica et Biophysica Acta*, vol. 1219, no. 2, pp. 533–535, 1994.

[36] S. O. Angel, J. M. Requena, M. Soto, D. Criado, and C. Alonso, "During canine leishmaniasis a protein belonging to the 83-kDa heat-shock protein family elicits a strong humoral response," *Acta Tropica*, vol. 62, no. 1, pp. 45–56, 1996.

[37] J. MacFarlane, M. L. Blaxter, R. P. Bishop, M. A. Miles, and J. M. Kelly, "Identification and characterisation of a *Leishmania donovani* antigen belonging to the 70-kDa heat-shock protein family," *European Journal of Biochemistry*, vol. 190, no. 2, pp. 377–384, 1990.

[38] J. M. Burns Jr., J. M. Scott, E. M. Carvalho, et al., "Characterization of a membrane antigen of *Leishmania amazonensis* that stimulates human immune responses," *The Journal of Immunology*, vol. 146, no. 2, pp. 742–748, 1991.

[39] Y. A. Skeiky, J. A. Guderian, D. R. Benson, et al., "A recombinant *Leishmania* antigen that stimulates human peripheral blood mononuclear cells to express a Th1-type cytokine profile and to produce interleukin 12," *Journal of Experimental Medicine*, vol. 181, no. 4, pp. 1527–1537, 1995.

[40] Y. A. Skeiky, D. R. Benson, J. A. Guderian, et al., "Immune responses of leishmaniasis patients to heat shock proteins of *Leishmania* species and humans," *Infection and Immunity*, vol. 63, no. 10, pp. 4105–4114, 1995.

[41] J. R. Webb, D. Kaufmann, A. Campos-Neto, and S. G. Reed, "Molecular cloning of a novel protein antigen of *Leishmania major* that elicits a potent immune response in experimental murine leishmaniasis," *The Journal of Immunology*, vol. 157, no. 11, pp. 5034–5041, 1996.

[42] N. Santarém, R. Silvestre, J. Tavares, et al., "Immune response regulation by *Leishmania* secreted and nonsecreted antigens," *Journal of Biomedicine and Biotechnology*, vol. 2007, Article ID 85154, 10 pages, 2007.

[43] J. M. Requena, C. Alonso, and M. Soto, "Evolutionarily conserved proteins as prominent immunogens during *Leishmania* infections," *Parasitology Today*, vol. 16, no. 6, pp. 246–250, 2000.

[44] F. Y. Liew and C. A. O'Donnell, "Immunology of Leishmaniasis," *Advances in Parasitology*, vol. 32, pp. 161–259, 1993.

[45] J. M. Requena, S. Iborra, J. Carrión, C. Alonso, and M. Soto, "Recent advances in vaccines for leishmaniasis," *Expert Opinion on Biological Therapy*, vol. 4, no. 9, pp. 1505–1517, 2004.

[46] R. M. Locksley and J. A. Louis, "Immunology of leishmaniasis," *Current Opinion in Immunology*, vol. 4, no. 4, pp. 413–418, 1992.

[47] P. Scott, P. Caspar, and A. Sher, "Protection against *Leishmania major* in BALB/c mice by adoptive transfer of a T cell clone recognizing a low molecular weight antigen released by promastigotes," *The Journal of Immunology*, vol. 144, no. 3, pp. 1075–1079, 1990.

[48] E. Mougneau, F. Altare, A. E. Wakil, et al., "Expression cloning of a protective *Leishmania* antigen," *Science*, vol. 268, no. 5210, pp. 563–566, 1995.

[49] P. Probst, E. Stromberg, H. W. Ghalib, et al., "Identification and characterization of T cell-stimulating antigens from *Leishmania* by CD4 T cell expression cloning," *The Journal of Immunology*, vol. 166, no. 1, pp. 498–505, 2001.

[50] S. Gurunathan, D. M. Klinman, and R. A. Seder, "DNA vaccines: immunology, application, and optimization*," *Annual Review of Immunology*, vol. 18, pp. 927–974, 2000.

[51] P. M. Smooker, A. Rainczuk, N. Kennedy, and T. W. Spithill, "DNA vaccines and their application against parasites—promise, limitations and potential solutions," *Biotechnology Annual Review*, vol. 10, pp. 189–236, 2004.

[52] D. Piedrafita, D. Xu, D. Hunter, R. A. Harrison, and F. Y. Liew, "Protective immune responses induced by vaccination with an expression genomic library of *Leishmania major*," *The Journal of Immunology*, vol. 163, no. 3, pp. 1467–1472, 1999.

[53] P. C. Melby, G. B. Ogden, H. A. Flores, et al., "Identification of vaccine candidates for experimental visceral leishmaniasis by immunization with sequential fractions of a cDNA expression library," *Infection and Immunity*, vol. 68, no. 10, pp. 5595–5602, 2000.

[54] C. B. Stober, U. G. Lange, M. T. M. Roberts, et al., "From genome to vaccines for leishmaniasis: screening 100 novel vaccine candidates against murine *Leishmania major* infection," *Vaccine*, vol. 24, no. 14, pp. 2602–2616, 2006.

[55] N. J. Fasel, D. C. Robyr, J. Mauel, and T. A. Glaser, "Identification of a histone H1-like gene expressed in *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 62, no. 2, pp. 321–324, 1993.

[56] R. M. R. Coulson and D. F. Smith, "Isolation of genes showing increased or unique expression in the infective promastigotes of *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 40, no. 1, pp. 63–75, 1990.

[57] H. Charest and G. Matlashewski, "Developmental gene expression in *Leishmania donovani*: differential cloning and analysis of an amastigote-stage-specific gene," *Molecular and Cellular Biology*, vol. 14, no. 5, pp. 2975–2984, 1994.

[58] B. Yahiaoui, A. Taibi, and A. Ouaissi, "A *Leishmania major* protein with extensive homology to silent information regulator 2 of *Saccharomyces cerevisiae*," *Gene*, vol. 169, no. 1, pp. 115–118, 1996.

[59] Y. Goto, R. F. Howard, A. Bhatia, et al., "Distinct antigen recognition pattern during zoonotic visceral leishmaniasis in humans and dogs," *Veterinary Parasitology*, vol. 160, no. 3-4, pp. 215–220, 2009.

[60] R. A. Frye, "Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins," *Biochemical and Biophysical Research Communications*, vol. 273, no. 2, pp. 793–798, 2000.

[61] S. M. Gasser and M. M. Cockell, "The molecular biology of the SIR proteins," *Gene*, vol. 279, no. 1, pp. 1–16, 2001.

[62] B. Vergnes, D. Sereno, N. Madjidian-Sereno, J.-L. Lemesre, and A. Ouaissi, "Cytoplasmic SIR2 homologue overexpression promotes survival of *Leishmania* parasites by preventing programmed cell death," *Gene*, vol. 296, no. 1-2, pp. 139–150, 2002.

[63] K. Zemzoumi, D. Sereno, C. François, E. Guilvard, J.-L. Lemesre, and A. Ouaissi, "*Leishmania major*: cell type dependent distribution of a 43 kDa antigen related to silent information regulatory-2 protein family," *Biology of the Cell*, vol. 90, no. 3, pp. 239–245, 1998.

[64] R. Silvestre, A. Cordeiro-da-Silva, J. Tavares, D. Sereno, and A. Ouaissi, "*Leishmania* cytosolic silent information regulatory protein 2 deacetylase induces murine B-cell differentiation and in vivo production of specific antibodies," *Immunology*, vol. 119, no. 4, pp. 529–540, 2006.

[65] A. Cordeiro-da-Silva, L. Cardoso, N. Araújo, et al., "Identification of antibodies to *Leishmania* silent information regulatory 2 (SIR2) protein homologue during canine natural infections: pathological implications," *Immunology Letters*, vol. 86, no. 2, pp. 155–162, 2003.

[66] N. Santarém, A. Tomás, A. Ouaissi, et al., "Antibodies against a *Leishmania infantum* peroxiredoxin as a possible marker for diagnosis of visceral leishmaniasis and for monitoring the efficacy of treatment," *Immunology Letters*, vol. 101, no. 1, pp. 18–23, 2005.

[67] P. F. P. Pimenta, E. M. B. Saraiva, and D. L. Sacks, "The comparative fine structure and surface glycoconjugate expression of three life stages of *Leishmania major*," *Experimental Parasitology*, vol. 72, no. 2, pp. 191–204, 1991.

[68] M. A. J. Ferguson, "The surface glycoconjugates of trypanosomatid parasites," *Philosophical Transactions of the Royal Society B*, vol. 352, no. 1359, pp. 1295–1302, 1997.

[69] M. J. McConville and J. M. Blackwell, "Developmental changes in the glycosylated phosphatidylinositols of *Leishmania donovani*. Characterization of the promastigote and amastigote glycolipids," *The Journal of Biological Chemistry*, vol. 266, no. 23, pp. 15170–15179, 1991.

[70] J. Bouvier, R. J. Etges, and C. Bordier, "Identification and purification of membrane and soluble forms of the major surface protein of *Leishmania* promastigotes," *The Journal of Biological Chemistry*, vol. 260, no. 29, pp. 15504–15509, 1985.

[71] C. Yao, J. E. Donelson, and M. E. Wilson, "The major surface protease (MSP or GP63) of *Leishmania* sp. Biosynthesis, regulation of expression, and function," *Molecular and Biochemical Parasitology*, vol. 132, no. 1, pp. 1–16, 2003.

[72] R. Etges, J. Bouvier, and C. Bordier, "The major surface protein of *Leishmania* promastigotes is a protease," *The Journal of Biological Chemistry*, vol. 261, no. 20, pp. 9098–9101, 1986.

[73] C. Bordier, "The promastigote surface protease of *Leishmania*," *Parasitology Today*, vol. 3, no. 5, pp. 151–153, 1987.

[74] J. Bouvier, R. Etges, and C. Bordier, "Identification of the promastigote surface protease in seven species of *Leishmania*," *Molecular and Biochemical Parasitology*, vol. 24, no. 1, pp. 73–79, 1987.

[75] E. A. Okong'o-Odera, J. A. L. Kurtzhals, A. S. Hey, and A. Kharazmi, "Measurement of serum antibodies against native *Leishmania* gp63 distinguishes between ongoing and previous *L. donovani* infection," *APMIS*, vol. 101, no. 8, pp. 642–646, 1993.

[76] W. G. Shreffler, J. M. Burns Jr., R. Badaró, et al., "Antibody responses of visceral leishmaniasis patients to gp63, a major surface glycoprotein of *Leishmania* species," *Journal of Infectious Diseases*, vol. 167, no. 2, pp. 426–430, 1993.

[77] G. Morales, G. Carrillo, J. M. Requena, et al., "Mapping of the antigenic determinants of the *Leishmania infantum* gp63 protein recognized by antibodies elicited during canine visceral leishmaniasis," *Parasitology*, vol. 114, part 6, pp. 507–516, 1997.

[78] T. Sanyal, D. K. Ghosh, and D. Sarkar, "Immunoblotting identifies an antigen recognized by anti gp63 in the immune complexes of Indian kala-azar patient sera," *Molecular and Cellular Biochemistry*, vol. 130, no. 1, pp. 11–17, 1994.

[79] T. Chakraborti, D. Sarkar, and D. K. Ghosh, "Immune complex antigens as a tool in serodiagnosis of kala-azar," *Molecular and Cellular Biochemistry*, vol. 253, no. 1-2, pp. 191–198, 2003.

[80] E. W. Mohareb, H. A. Hanafi, E. M. Mikhail, S. M. Presley, and R. Batchelor, "Evaluation of an indirect immunofluorescence assay for the detection of *Leishmania* promastigotes and amastigotes in sand flies and lesion fluid aspirates," *Journal of the Egyptian Society of Parasitology*, vol. 28, no. 2, pp. 313–321, 1998.

[81] L. P. Kahl, R. Lelchuk, C. A. Scott, and J. Beesley, "Characterization of *Leishmania major* antigen-liposomes that protect BALB/c mice against cutaneous leishmaniasis," *Infection and Immunity*, vol. 58, no. 10, pp. 3233–3241, 1990.

[82] D. G. Russell and J. Alexander, "Effective immunization cutaneous leishmaniasis with defined membrane antigens reconsituted into liposomes," *The Journal of Immunology*, vol. 140, no. 4, pp. 1274–1279, 1988.

[83] F. Afrin, R. Rajesh, K. Anam, M. Gopinath, S. Pal, and N. Ali, "Characterization of *Leishmania donovani* antigens encapsulated in liposomes that induce protective immunity in BALB/c mice," *Infection and Immunity*, vol. 70, no. 12, pp. 6697–6706, 2002.

[84] S. Bhowmick, R. Ravindran, and N. Ali, "gp63 in stable cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with *Leishmania donovani*," *Infection and Immunity*, vol. 76, no. 3, pp. 1003–1015, 2008.

[85] M. R. Jaafari, A. Ghafarian, A. Farrokh-Gisour, et al., "Immune response and protection assay of recombinant

major surface glycoprotein of *Leishmania* (rgp63) reconstituted with liposomes in BALB/c mice," *Vaccine*, vol. 24, no. 29-30, pp. 5708–5717, 2006.

[86] M. R. Jaafari, A. Badiee, A. Khamesipour, et al., "The role of CpG ODN in enhancement of immune response and protection in BALB/c mice immunized with recombinant major surface glycoprotein of *Leishmania* (rgp63) encapsulated in cationic liposome," *Vaccine*, vol. 25, no. 32, pp. 6107–6117, 2007.

[87] D. M. Yang, N. Fairweather, L. L. Button, W. R. McMaster, L. P. Kahl, and F. Y. Liew, "Oral Salmonella typhimurium (AroA-) vaccine expressing a major leishmanial surface protein (gp63) preferentially induces T helper 1 cells and protective immunity against leishmaniasis," *The Journal of Immunology*, vol. 145, no. 7, pp. 2281–2285, 1990.

[88] S. Abdelhak, H. Louzir, J. Timm, et al., "Recombinant BCG expressing the *Leishmania* surface antigen gp63 induces protective immunity against *Leishmania major* infection in BALB/c mice," *Microbiology*, vol. 141, part 7, pp. 1585–1592, 1995.

[89] N. D. Connell, E. Medina-Acosta, W. R. McMaster, B. R. Bloom, and D. G. Russell, "Effective immunization against cutaneous leishmaniasis with recombinant bacille Calmette-Guérin expressing the *Leishmania* surface proteinase gp63," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 24, pp. 11473–11477, 1993.

[90] C. W. Xu, J. C. Hines, M. L. Engel, D. G. Russell, and D. S. Ray, "Nucleus-encoded histone H1-like proteins are associated with kinetoplast DNA in the trypanosomatid *Crithidia fasciculata*," *Molecular and Cellular Biology*, vol. 16, no. 2, pp. 564–576, 1996.

[91] S. Frankenburg, O. Axelrod, S. Kutner, et al., "Effective immunization of mice against cutaneous leishmaniasis using an intrinsically adjuvanted synthetic lipopeptide vaccine," *Vaccine*, vol. 14, no. 9, pp. 923–929, 1996.

[92] P. Tsagozis, E. Karagouni, and E. Dotsika, "Dendritic cells pulsed with peptides of gp63 induce differential protection against experimental cutaneous leishmaniasis," *International Journal of Immunopathology and Pharmacology*, vol. 17, no. 3, pp. 343–352, 2004.

[93] D. McMahon-Pratt, Y. Traub-Cseko, K. L. Lohman, D. D. Rogers, and S. M. Beverley, "Loss of the GP46/M-2 surface membrane glycoprotein gene family in the *Leishmania braziliensis* complex," *Molecular and Biochemical Parasitology*, vol. 50, no. 1, pp. 151–160, 1992.

[94] L. P. Kahl and D. McMahon-Pratt, "Structural and antigenic characterization of a species- and promastigote-specific *Leishmania mexicana* amazonensis membrane protein," *The Journal of Immunology*, vol. 138, no. 5, pp. 1587–1595, 1987.

[95] I. A. Maalej, M. Chenik, H. Louzir, et al., "Comparative evaluation of ELISAs based on ten recombinant or purified *Leishmania* antigens for the serodiagnosis of Mediterranean visceral leishmaniasis," *The American Journal of Tropical Medicine and Hygiene*, vol. 68, no. 3, pp. 312–320, 2003.

[96] C. Boceta, C. Alonso, and A. Jiménez-Ruiz, "Leucine rich repeats are the main epitopes in *Leishmania infantum* PSA during canine and human visceral leishmaniasis," *Parasite Immunology*, vol. 22, no. 2, pp. 55–62, 2000.

[97] E. Handman, F. M. Symons, T. M. Baldwin, J. M. Curtis, and J.-P. Y. Scheerlinck, "Protective vaccination with promastigote surface antigen 2 from *Leishmania major* is mediated by a TH1 type of immune response," *Infection and Immunity*, vol. 63, no. 11, pp. 4261–4267, 1995.

[98] A. Sjölander, T. M. Baldwin, J. M. Curtis, and E. Handman, "Induction of a Th1 immune response and simultaneous lack of activation of a Th2 response are required for generation of immunity to Leishmaniasis," *The Journal of Immunology*, vol. 160, no. 8, pp. 3949–3957, 1998.

[99] A. Sjölander, T. M. Baldwin, J. M. Curtis, K. L. Bengtsson, and E. Handman, "Vaccination with recombinant Parasite Surface Antigen 2 from *Leishmania major* induces a Th1 type of immune response but does not protect against infection," *Vaccine*, vol. 16, no. 20, pp. 2077–2084, 1998.

[100] E. Handman, A. H. Noormohammadi, J. M. Curtis, T. Baldwin, and A. Sjölander, "Therapy of murine cutaneous leishmaniasis by DNA vaccination," *Vaccine*, vol. 18, no. 26, pp. 3011–3017, 2000.

[101] E. Dumonteil, R.-S. Maria Jesus, E.-O. Javier, and G.-M. Maria del Rosario, "DNA vaccines induce partial protection against *Leishmania mexicana*," *Vaccine*, vol. 21, no. 17-18, pp. 2161–2168, 2003.

[102] E. Dumonteil, F. Andrade-Narvarez, J. Escobedo-Ortegon, et al., "Comparative study of DNA vaccines encoding various antigens against *Leishmania mexicana*," *Developments in Biologicals*, vol. 104, pp. 135–141, 2000.

[103] D. McMahon-Pratt, D. Rodriguez, J.-R. Rodriguez, et al., "Recombinant vaccinia viruses expressing GP46/M-2 protect against *Leishmania* infection," *Infection and Immunity*, vol. 61, no. 8, pp. 3351–3359, 1993.

[104] D. L. Tolson, A. Jardim, L. F. Schnur, et al., "The kinetoplastid membrane protein 11 of *Leishmania donovani* and African trypanosomes is a potent stimulator of T-lymphocyte proliferation," *Infection and Immunity*, vol. 62, no. 11, pp. 4893–4899, 1994.

[105] A. Jardim, V. Funk, R. M. Caprioli, and R. W. Olafson, "Isolation and structural characterization of the *Leishmania donovani* kinetoplastid membrane protein-11, a major immunoreactive membrane glycoprotein," *Biochemical Journal*, vol. 305, part 1, pp. 307–313, 1995.

[106] C. Berberich, J. M. Requena, and C. Alonso, "Cloning of genes and expression and antigenicity analysis of the *Leishmania infantum* KMP-11 protein," *Experimental Parasitology*, vol. 85, no. 1, pp. 105–108, 1997.

[107] J. R. Ramírez, C. Berberich, A. Jaramillo, C. Alonso, and I. D. Vélez, "Molecular and antigenic characterization of the *Leishmania (Viannia) panamensis* kinetoplastid membrane protein-11," *Memórias do Instituto Oswaldo Cruz*, vol. 93, no. 2, pp. 247–254, 1998.

[108] A. T. R. Jensen, S. Gasim, A. Ismail, et al., "Humoral and cellular immune responses to synthetic peptides of the *Leishmania donovani* kinetoplastid membrane protein-11," *Scandinavian Journal of Immunology*, vol. 48, no. 1, pp. 103–109, 1998.

[109] C. Trujillo, R. Ramírez, I. D. Vélez, and C. Berberich, "The humoral immune response to the kinetoplastid membrane protein-11 in patients with American Leishmaniasis and Chagas disease: prevalence of IgG subclasses and mapping of epitopes," *Immunology Letters*, vol. 70, no. 3, pp. 203–209, 2000.

[110] S. Passos, L. P. de Carvalho, G. Orge, et al., "Recombinant *Leishmania* antigens for serodiagnosis of visceral leishmaniasis," *Clinical and Diagnostic Laboratory Immunology*, vol. 12, no. 10, pp. 1164–1167, 2005.

[111] A. Jardim, D. L. Tolson, S. J. Turco, T. W. Pearson, and R. W. Olafson, "The *Leishmania donovani* lipophosphoglycan T lymphocyte-reactive component is a tightly associated

protein complex," *The Journal of Immunology*, vol. 147, no. 10, pp. 3538–3544, 1991.

[112] E. Carrillo, M. Crusat, J. Nieto, et al., "Immunogenicity of HSP-70, KMP-11 and PFR-2 leishmanial antigens in the experimental model of canine visceral leishmaniasis," *Vaccine*, vol. 26, no. 15, pp. 1902–1911, 2008.

[113] R. Basu, S. Bhaumik, J. M. Basu, K. Naskar, T. De, and S. Roy, "Kinetoplastid membrane protein-11 DNA vaccination induces complete protection against both pentavalent antimonial-sensitive and -resistant strains of *Leishmania donovani* that correlates with inducible nitric oxide synthase activity and IL-4 generation: evidence for mixed Th1- and Th2-like responses in visceral leishmaniasis," *The Journal of Immunology*, vol. 174, no. 11, pp. 7160–7171, 2005.

[114] J. R. Ramírez, K. Gilchrist, S. Robledo, et al., "Attenuated *Toxoplasma gondii* ts-4 mutants engineered to express the *Leishmania* antigen KMP-11 elicit a specific immune response in BALB/c mice," *Vaccine*, vol. 20, no. 3-4, pp. 455–461, 2001.

[115] P. G. McKean, R. Delahay, P. F. P. Pimenta, and D. F. Smith, "Characterisation of a second protein encoded by the differentially regulated LmcDNA16 gene family of *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 85, no. 2, pp. 221–231, 1997.

[116] H. M. Flinn, D. Rangarajan, and D. F. Smith, "Expression of a hydrophilic surface protein in infective stages of *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 65, no. 2, pp. 259–270, 1994.

[117] T. M. Alce, S. Gokool, D. McGhie, S. Stäger, and D. F. Smith, "Expression of hydrophilic surface proteins in infective stages of *Leishmania donovani*," *Molecular and Biochemical Parasitology*, vol. 102, no. 1, pp. 191–196, 1999.

[118] A. T. R. Jensen, S. Gasim, T. Moller, et al., "Serodiagnosis of *Leishmania donovani* infections: assessment of enzyme-linked immunosorbent assays using recombinant *L. donovani* gene B protein (GBP) and a peptide sequence of *L. donovani* GBP," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 93, no. 2, pp. 157–160, 1999.

[119] S. Stäger, D. F. Smith, and P. M. Kaye, "Immunization with a recombinant stage-regulated surface protein from *Leishmania donovani* induces protection against visceral leishmaniasis," *The Journal of Immunology*, vol. 165, no. 12, pp. 7064–7071, 2000.

[120] J. Moreno, J. Nieto, S. Masina, et al., "Immunization with H1, HASPB1 and MML *Leishmania* proteins in a vaccine trial against experimental canine leishmaniasis," *Vaccine*, vol. 25, no. 29, pp. 5290–5300, 2007.

[121] R. Badaró, D. Benson, M. C. Eulálio, et al., "rK39: a cloned antigen of *Leishmania* chagasi that predicts active visceral leishmaniasis," *The Journal of Infectious Diseases*, vol. 173, no. 3, pp. 758–761, 1996.

[122] K. G. J. Pollock, K. S. McNeil, J. C. Mottram, et al., "The *Leishmania mexicana* cysteine protease, CPB2.8, induces potent Th2 responses," *The Journal of Immunology*, vol. 170, no. 4, pp. 1746–1753, 2003.

[123] S. Rafati, A. Nakhaee, T. Taheri, et al., "Expression of cysteine proteinase type I and II of *Leishmania infantum* and their recognition by sera during canine and human visceral leishmaniasis," *Experimental Parasitology*, vol. 103, no. 3-4, pp. 143–151, 2003.

[124] E. Pateraki, R. Portocala, H. Labrousse, and J. L. Guesdon, "Antiactin and antitubulin antibodies in canine visceral leishmaniasis," *Infection and Immunity*, vol. 42, no. 2, pp. 496–500, 1983.

[125] K. Zemzoumi, E. Guilvard, D. Sereno, et al., "Cloning of a *Leishmania major* gene encoding for an antigen with extensive homology to ribosomal protein S3a," *Gene*, vol. 240, no. 1, pp. 57–65, 1999.

[126] Y. A. W. Skeiky, D. R. Benson, M. Elwasila, R. Badaro, J. M. Burns Jr., and S. G. Reed, "Antigens shared by *Leishmania* species and *Trypanosoma cruzi*: immunological comparison of the acidic ribosomal P0 proteins," *Infection and Immunity*, vol. 62, no. 5, pp. 1643–1651, 1994.

[127] A. M. Montalvo-Álvarez, C. Folgueira, J. Carrión, L. Monzote-Fidalgo, C. Cañavate, and J. M. Requena, "The *Leishmania* HSP20 is antigenic during natural infections, but, as DNA vaccine, it does not protect BALB/c mice against experimental *L. amazonensis* infection," *Journal of Biomedicine and Biotechnology*, vol. 2008, Article ID 695432, 9 pages, 2008.

[128] J. A. Rey-Ladino, P. B. Joshi, B. Singh, R. Gupta, and N. E. Reiner, "*Leishmania major*: molecular cloning, sequencing, and expression of the heat shock protein 60 gene reveals unique carboxy terminal peptide sequences," *Experimental Parasitology*, vol. 85, no. 3, pp. 249–263, 1997.

[129] L. Quijada, J. M. Requena, M. Soto, and C. Alonso, "During canine viscero-cutaneous leishmaniasis the anti-Hsp70 antibodies are specifically elicited by the parasite protein," *Parasitology*, vol. 112, part 3, pp. 277–284, 1996.

[130] R. Larreta, M. Soto, C. Alonso, and J. M. Requena, "*Leishmania infantum*: gene cloning of the GRP94 homologue, its expression as recombinant protein, and analysis of antigenicity," *Experimental Parasitology*, vol. 96, no. 2, pp. 108–115, 2000.

[131] M. Soto, J. M. Requena, L. Quijada, et al., "Antigenicity of the *Leishmania infantum* histones H2B and H4 during canine viscerocutaneous leishmaniasis," *Clinical & Experimental Immunology*, vol. 115, no. 2, pp. 342–349, 1999.

[132] S. Rafati, A.-H. Salmanian, K. Hashemi, C. Schaff, S. Belli, and N. Fasel, "Identification of *Leishmania major* cysteine proteinases as targets of the immune response in humans," *Molecular and Biochemical Parasitology*, vol. 113, no. 1, pp. 35–43, 2001.

[133] N. Khoshgoo, F. Zahedifard, H. Azizi, Y. Taslimi, M. J. Alonso, and S. Rafati, "Cysteine proteinase type III is protective against *Leishmania infantum* infection in BALB/c mice and highly antigenic in visceral leishmaniasis individuals," *Vaccine*, vol. 26, no. 46, pp. 5822–5829, 2008.

[134] J. M. Burns Jr., W. G. Shreffler, D. R. Benson, H. W. Ghalib, R. Badaro, and S. G. Reed, "Molecular characterization of a kinesin-related antigen of *Leishmania chagasi* that detects specific antibody in African and American visceral leishmaniasis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 2, pp. 775–779, 1993.

[135] M. T. M. Roberts, C. B. Stober, A. N. McKenzie, and J. M. Blackwell, "Interleukin-4 (IL-4) and IL-10 collude in vaccine failure for novel exacerbatory antigens in murine *Leishmania major* infection," *Infection and Immunity*, vol. 73, no. 11, pp. 7620–7628, 2005.

[136] A. Cordeiro-da-Silva, M. C. Borges, E. Guilvard, and A. Ouaissi, "Dual role of the *Leishmania major* ribosomal protein S3a homologue in regulation of T- and B-cell activation," *Infection and Immunity*, vol. 69, no. 11, pp. 6588–6596, 2001.

[137] M. Soto, J. M. Requena, L. Quijada, F. Guzman, M. E. Patarroyo, and C. Alonso, "Identification of the *Leishmania infantum* P0 ribosomal protein epitope in canine visceral

leishmaniasis," *Immunology Letters*, vol. 48, no. 1, pp. 23–28, 1995.

[138] M. Soto, J. M. Requena, L. Quijada, et al., "During active viscerocutaneous leishmaniasis the anti-P2 humoral response is specifically triggered by the parasite P proteins," *Clinical & Experimental Immunology*, vol. 100, no. 2, pp. 246–252, 1995.

[139] M. J. Levin, M. Vazquez, D. Kaplan, and A. G. Schijman, "The *Trypanosoma cruzi* ribosomal P protein family: classification and antigenicity," *Parasitology Today*, vol. 9, no. 10, pp. 381–384, 1993.

[140] S. Iborra, D. R. Abánades, N. Parody, et al., "The immunodominant T helper 2 (Th2) response elicited in BALB/c mice by the *Leishmania* LiP2a and LiP2b acidic ribosomal proteins cannot be reverted by strong Th1 inducers," *Clinical & Experimental Immunology*, vol. 150, no. 2, pp. 375–385, 2007.

[141] M. Soto, C. Alonso, and J. M. Requena, "The *Leishmania infantum* acidic ribosomal protein LiP2a induces a prominent humoral response in vivo and stimulates cell proliferation in vitro and interferon-gamma (IFN-γ) production by murine splenocytes," *Clinical & Experimental Immunology*, vol. 122, no. 2, pp. 212–218, 2000.

[142] M. Soto, J. M. Requena, L. Quijada, and C. Alonso, "Specific serodiagnosis of human leishmaniasis with recombinant *Leishmania* P2 acidic ribosomal proteins," *Clinical and Diagnostic Laboratory Immunology*, vol. 3, no. 4, pp. 387–391, 1996.

[143] K. Elkon, S. Skelly, A. Parnassa, et al., "Identification and chemical synthesis of a ribosomal protein antigenic determinant in systemic lupus erythematosus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 83, no. 19, pp. 7419–7423, 1986.

[144] C. Mayer, U. Appenzeller, H. Seelbach, et al., "Humoral and cell-mediated autoimmune reactions to human acidic ribosomal P_2 protein in individuals sensitized to *Aspergillus fumigatus* P_2 protein," *Journal of Experimental Medicine*, vol. 189, no. 9, pp. 1507–1512, 1999.

[145] Y. A. W. Skeiky, D. R. Benson, M. Parsons, K. B. Elkon, and S. G. Reed, "Cloning and expression of *Trypanosoma cruzi* ribosomal protein P0 and epitope analysis of anti-P0 autoantibodies in Chagas' disease patients," *Journal of Experimental Medicine*, vol. 176, no. 1, pp. 201–211, 1992.

[146] S. Iborra, M. Soto, J. Carrión, et al., "The *Leishmania infantum* acidic ribosomal protein P0 administered as a DNA vaccine confers protective immunity to *Leishmania major* infection in BALB/c mice," *Infection and Immunity*, vol. 71, no. 11, pp. 6562–6572, 2003.

[147] S. Iborra, J. Carrión, C. Anderson, C. Alonso, D. Sacks, and M. Soto, "Vaccination with the *Leishmania infantum* acidic ribosomal P0 protein plus CpG oligodeoxynucleotides induces protection against cutaneous leishmaniasis in C57BL/6 mice but does not prevent progressive disease in BALB/c mice," *Infection and Immunity*, vol. 73, no. 9, pp. 5842–5852, 2005.

[148] E. Arndt and C. Weigel, "Nucleotide sequence of the genes encoding the L11, L1, L10 and L12 equivalent ribosomal proteins from the archaebacterium *Halobacterium marismortui*," *Nucleic Acids Research*, vol. 18, no. 5, p. 1285, 1990.

[149] D. B. Young, "Heat-shock proteins: immunity and autoimmunity," *Current Opinion in Immunology*, vol. 4, no. 4, pp. 396–400, 1992.

[150] C. Folgueira and J. M. Requena, "A postgenomic view of the heat shock proteins in kinetoplastids," *FEMS Microbiology Reviews*, vol. 31, no. 4, pp. 359–377, 2007.

[151] B. J. Celeste, S. O. Angel, L. G. M. Castro, M. Gidlund, and H. Goto, "*Leishmania infantum* heat shock protein 83 for the serodiagnosis of tegumentary leishmaniasis," *Brazilian Journal of Medical and Biological Research*, vol. 37, no. 11, pp. 1591–1593, 2004.

[152] S. K. Gupta, B. S. Sisodia, S. Sinha, et al., "Proteomic approach for identification and characterization of novel immunostimulatory proteins from soluble antigens of *Leishmania donovani* promastigotes," *Proteomics*, vol. 7, no. 5, pp. 816–823, 2007.

[153] A. Descoteaux, H. A. Avila, K. Zhang, S. J. Turco, and S. M. Beverley, "*Leishmania LPG3* encodes a GRP94 homolog required for phosphoglycan synthesis implicated in parasite virulence but not viability," *The EMBO Journal*, vol. 21, no. 17, pp. 4458–4469, 2002.

[154] R. Larreta, F. Guzman, M. E. Patarroyo, C. Alonso, and J. M. Requena, "Antigenic properties of the *Leishmania infantum* GRP94 and mapping of linear B-cell epitopes," *Immunology Letters*, vol. 80, no. 3, pp. 199–205, 2002.

[155] L. Quijada, J. M. Requena, M. Soto, and C. Alonso, "Analysis of the antigenic properties of the *L. infantum* Hsp70: design of synthetic peptides for specific serodiagnosis of human leishmaniasis," *Immunology Letters*, vol. 63, no. 3, pp. 169–174, 1998.

[156] A. I. Zurita, J. Rodríguez, J. E. Piñero, et al., "Cloning and characterization of the *Leishmania (Viannia) braziliensis HSP70* gene. Diagnostic use of the C-terminal fragment rlb70(513–663)," *Journal of Parasitology*, vol. 89, no. 2, pp. 372–378, 2003.

[157] P. Echeverria, G. Dran, G. Pereda, et al., "Analysis of the adjuvant effect of recombinant *Leishmania infantum* Hsp83 protein as a tool for vaccination," *Immunology Letters*, vol. 76, no. 2, pp. 107–110, 2001.

[158] A. I. Rico, G. Del Real, M. Soto, et al., "Characterization of the immunostimulatory properties of *Leishmania infantum* HSP70 by fusion to the *Escherichia coli* maltose-binding protein in normal and *nu/nu* BALB/c mice," *Infection and Immunity*, vol. 66, no. 1, pp. 347–352, 1998.

[159] S. Rafati, E. Gholami, N. Hassani, et al., "*Leishmania major* heat shock protein 70 (HSP70) is not protective in murine models of cutaneous leishmaniasis and stimulates strong humoral responses in cutaneous and visceral leishmaniasis patients," *Vaccine*, vol. 25, no. 21, pp. 4159–4169, 2007.

[160] J. R. Webb, A. Campos-Neto, Y. A. W. Skeiky, and S. G. Reed, "Molecular characterization of the heat-inducible LmSTI1 protein of *Leishmania major*," *Molecular and Biochemical Parasitology*, vol. 89, no. 2, pp. 179–193, 1997.

[161] A. Campos-Neto, R. Porrozzi, K. Greeson, et al., "Protection against cutaneous leishmaniasis induced by recombinant antigens in murine and nonhuman primate models of the human disease," *Infection and Immunity*, vol. 69, no. 6, pp. 4103–4108, 2001.

[162] A. Campos-Neto, J. R. Webb, K. Greeson, R. N. Coler, Y. A. W. Skeiky, and S. G. Reed, "Vaccination with plasmid DNA encoding TSA/LmSTI1 Leishmanial fusion proteins confers protection against *Leishmania major* infection in susceptible BALB/c mice," *Infection and Immunity*, vol. 70, no. 6, pp. 2828–2836, 2002.

[163] A. Badiee, M. R. Jaafari, A. Samiei, D. Soroush, and A. Khamesipour, "Coencapsulation of CpG oligodeoxynucleotides with recombinant *Leishmania major* stress-inducible protein 1 in liposome enhances immune response and protection against leishmaniasis in immunized BALB/c

mice," *Clinical and Vaccine Immunology*, vol. 15, no. 4, pp. 668–674, 2008.

[164] M. Soto, J. M. Requena, L. Quijada, et al., "Mapping of the linear antigenic determinants from the *Leishmania infantum* histone H2A recognized by sera from dogs with leishmaniasis," *Immunology Letters*, vol. 48, no. 3, pp. 209–214, 1995.

[165] M. Soto, J. M. Requena, L. Quijada, et al., "Characterization of the antigenic determinants of the *Leishmania infantum* histone H3 recognized by antibodies elicited during canine visceral leishmaniasis," *Clinical & Experimental Immunology*, vol. 106, no. 3, pp. 454–461, 1996.

[166] Y. Montoya, C. Leon, M. Talledo, et al., "Recombinant antigens for specific and sensitive serodiagnosis of Latin American tegumentary leishmaniasis," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 91, no. 6, pp. 674–676, 1997.

[167] L. P. de Carvalho, M. Soto, S. Jerônimo, et al., "Characterization of the immune response to *Leishmania infantum* recombinant antigens," *Microbes and Infection*, vol. 5, no. 1, pp. 7–12, 2003.

[168] S. Iborra, M. Soto, J. Carrión, C. Alonso, and J. M. Requena, "Vaccination with a plasmid DNA cocktail encoding the nucleosomal histones of *Leishmania* confers protection against murine cutaneous leishmaniosis," *Vaccine*, vol. 22, no. 29-30, pp. 3865–3876, 2004.

[169] J. Carrión, A. Nieto, M. Soto, and C. Alonso, "Adoptive transfer of dendritic cells pulsed with *Leishmania infantum* nucleosomal histones confers protection against cutaneous leishmaniosis in BALB/c mice," *Microbes and Infection*, vol. 9, no. 6, pp. 735–743, 2007.

[170] J. Carrión, C. Folgueira, and C. Alonso, "Transitory or long-lasting immunity to *Leishmania major* infection: the result of immunogenicity and multicomponent properties of histone DNA vaccines," *Vaccine*, vol. 26, no. 9, pp. 1155–1165, 2008.

[171] J. Carrión, C. Folgueira, and C. Alonso, "Immunization strategies against visceral leishmaniosis with the nucleosomal histones of *Leishmania infantum* encoded in DNA vaccine or pulsed in dendritic cells," *Vaccine*, vol. 26, no. 20, pp. 2537–2544, 2008.

[172] M. Chenik, H. Louzir, H. Ksontini, A. Dilou, I. Abdmouleh, and K. Dellagi, "Vaccination with the divergent portion of the protein histone H2B of *Leishmania* protects susceptible BALB/c mice against a virulent challenge with *Leishmania major*," *Vaccine*, vol. 24, no. 14, pp. 2521–2529, 2006.

[173] D. Smirlis, S. N. Bisti, E. Xingi, G. Konidou, M. Thiakaki, and K. P. Soteriadou, "*Leishmania* histone H1 overexpression delays parasite cell-cycle progression, parasite differentiation and reduces *Leishmania* infectivity in vivo," *Molecular Microbiology*, vol. 60, no. 6, pp. 1457–1473, 2006.

[174] F. T. Papageorgiou and K. P. Soteriadou, "Expression of a novel *Leishmania* gene encoding a histone H1-like protein in *Leishmania major* modulates parasite infectivity in vitro," *Infection and Immunity*, vol. 70, no. 12, pp. 6976–6986, 2002.

[175] N. Solioz, U. Blum-Tirouvanziam, R. Jacquet, et al., "The protective capacities of histone H1 against experimental murine cutaneous leishmaniasis," *Vaccine*, vol. 18, no. 9-10, pp. 850–859, 1999.

[176] S. Masina, M. M. Gicheru, S. O. Demotz, and N. J. Fasel, " Protection against cutaneous leishmaniasis in outbred vervet monkeys using a recombinant histone H1 antigen," *The Journal of Infectious Diseases*, vol. 188, no. 8, pp. 1250–1257, 2003.

[177] J. H. McKerrow, E. Sun, P. J. Rosenthal, and J. Bouvier, "The proteases and pathogenicity of parasitic protozoa," *Annual Review of Microbiology*, vol. 47, pp. 821–853, 1993.

[178] J. A. Sakanari, S. A. Nadler, V. J. Chan, J. C. Engel, C. Leptak, and J. Bouvier, "*Leishmania major*: comparison of the cathepsin L- and B-like cysteine protease genes with those of other trypanosomatids," *Experimental Parasitology*, vol. 85, no. 1, pp. 63–76, 1997.

[179] J. C. Mottram, D. R. Brooks, and G. H. Coombs, "Roles of cysteine proteinases of trypanosomes and *Leishmania* in host-parasite interactions," *Current Opinion in Microbiology*, vol. 1, no. 4, pp. 455–460, 1998.

[180] J. C. Mottram, A. E. Souza, J. E. Hutchison, R. Carter, M. J. Frame, and G. H. Coombs, "Evidence from disruption of the lmcpb gene array of *Leishmania mexicana* that cysteine proteinases are virulence factors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 12, pp. 6008–6013, 1996.

[181] L. U. Buxbaum, H. Denise, G. H. Coombs, J. Alexander, J. C. Mottram, and P. Scott, "Cysteine protease B of *Leishmania mexicana* inhibits host Th1 responses and protective immunity," *The Journal of Immunology*, vol. 171, no. 7, pp. 3711–3717, 2003.

[182] J. Alexander and K. Bryson, "T helper (h)1/Th2 and *Leishmania*: paradox rather than paradigm," *Immunology Letters*, vol. 99, no. 1, pp. 17–23, 2005.

[183] S. de Souza Dias, P. H. da Costa Pinheiro, S. Katz, M. R. Machado dos Santos, and C. L. Barbiéri, "A recombinant cysteine proteinase from *Leishmania* (*Leishmania*) *chagasi* suitable for serodiagnosis of American visceral leishmaniasis," *The American Journal of Tropical Medicine and Hygiene*, vol. 72, no. 2, pp. 126–132, 2005.

[184] H. Pascalis, A. Lavergne, E. Bourreau, et al., "Th1 cell development induced by cysteine proteinases A and B in localized cutaneous leishmaniasis due to *Leishmania guyanensis*," *Infection and Immunity*, vol. 71, no. 5, pp. 2924–2926, 2003.

[185] A. Zadeh-Vakili, T. Taheri, Y. Taslimi, F. Doustdari, A.-H. Salmanian, and S. Rafati, "Immunization with the hybrid protein vaccine, consisting of *Leishmania major* cysteine proteinases Type I (CPB) and Type II (CPA), partially protects against leishmaniasis," *Vaccine*, vol. 22, no. 15-16, pp. 1930–1940, 2004.

[186] P. H. da Costa Pinheiro, S. de Souza Dias, K. Dantas Eulálio, I. L. Mendonça, S. Katz, and C. L. Barbiéri, "Recombinant cysteine proteinase from *Leishmania* (*Leishmania*) *chagasi* implicated in human and dog T-cell responses," *Infection and Immunity*, vol. 73, no. 6, pp. 3787–3789, 2005.

[187] S. Rafati, A.-H. Salmanian, T. Taheri, M. Vafa, and N. Fasel, "A protective cocktail vaccine against murine cutaneous leishmaniasis with DNA encoding cysteine proteinases of *Leishmania major*," *Vaccine*, vol. 19, no. 25-26, pp. 3369–3375, 2001.

[188] S. Rafati, A. Kariminia, S. Seyde-Eslami, M. Narimani, T. Taheri, and M. Lebbatard, "Recombinant cysteine proteinases-based vaccines against *Leishmania major* in BALB/c mice: the partial protection relies on interferon gamma producing $CD8^+$ T lymphocyte activation," *Vaccine*, vol. 20, no. 19-20, pp. 2439–2447, 2002.

[189] S. Rafati, F. Zahedifard, and F. Nazgouee, "Prime-boost vaccination using cysteine proteinases type I and II of *Leishmania infantum* confers protective immunity in murine visceral leishmaniasis," *Vaccine*, vol. 24, no. 12, pp. 2169–2175, 2006.

[190] S. Rafati, A. Nakhaee, T. Taheri, et al., "Protective vaccination against experimental canine visceral leishmaniasis using a combination of DNA and protein immunization with cysteine proteinases type I and II of *L. infantum*," *Vaccine*, vol. 23, no. 28, pp. 3716–3725, 2005.

[191] J. Poot, K. Spreeuwenberg, S. J. Sanderson, et al., "Vaccination with a preparation based on recombinant cysteine peptidases and canine IL-12 does not protect dogs from infection with *Leishmania infantum*," *Vaccine*, vol. 24, no. 14, pp. 2460–2468, 2006.

[192] P. Probst, Y. A. W. Skeiky, M. Steeves, A. Gervassi, K. H. Grabstein, and S. G. Reed, "A *Leishmania* protein that modulates interleukin (IL)-12, IL-10 and tumor necrosis factor-α production and expression of B7-1 in human monocyte-derived antigen-presenting cells," *European Journal of Immunology*, vol. 27, no. 10, pp. 2634–2642, 1997.

[193] Y. A. W. Skeiky, M. Kennedy, D. Kaufman, et al., "LeIF: a recombinant *Leishmania* protein that induces an IL-12-mediated Th1 cytokine profile," *The Journal of Immunology*, vol. 161, no. 11, pp. 6171–6179, 1998.

[194] M. M. Borges, A. Campos-Neto, P. Sleath, et al., "Potent stimulation of the innate immune system by a *Leishmania* brasiliensis recombinant protein," *Infection and Immunity*, vol. 69, no. 9, pp. 5270–5277, 2001.

[195] P. Launois, I. Maillard, S. Pingel, et al., "IL-4 rapidly produced by Vβ4 V α8 CD4$^+$ T cells instructs Th2 development and susceptibility to *Leishmania major* in BALB/c mice," *Immunity*, vol. 6, no. 5, pp. 541–549, 1997.

[196] S. Gurunathan, D. L. Sacks, D. R. Brown, et al., "Vaccination with DNA encoding the immunodominant LACK parasite antigen confers protective immunity to mice infected with *Leishmania major*," *Journal of Experimental Medicine*, vol. 186, no. 7, pp. 1137–1147, 1997.

[197] V. Julia, M. Rassoulzadegan, and N. Glaichenhaus, "Resistance to *Leishmania major* induced by tolerance to a single antigen," *Science*, vol. 274, no. 5286, pp. 421–423, 1996.

[198] S. Ben Hadj Ahmed, C. Bahloul, C. Robbana, S. Askri, and K. Dellagi, "A comparative evaluation of different DNA vaccine candidates against experimental murine leishmaniasis due to *L. major*," *Vaccine*, vol. 22, no. 13-14, pp. 1631–1639, 2004.

[199] N. Soussi, H. Saklani-Jusforgues, J.-H. Colle, G. Milon, N. Glaichenhaus, and P. L. Goossens, "Effect of intragastric and intraperitoneal immunisation with attenuated and wild-type LACK-expressing *Listeria monocytogenes* on control of murine *Leishmania major* infection," *Vaccine*, vol. 20, no. 21-22, pp. 2702–2712, 2002.

[200] R. M. Gonzalo, J. R. Rodríguez, D. Rodríguez, G. González-Aseguinolaza, V. Larraga, and M. Esteban, "Protective immune response against cutaneous leishmaniasis by prime/booster immunization regimens with vaccinia virus recombinants expressing *Leishmania infantum* p36/LACK and IL-12 in combination with purified p36," *Microbes and Infection*, vol. 3, no. 9, pp. 701–711, 2001.

[201] R. M. Gonzalo, G. del Real, J. R. Rodriguez, et al., "A heterologous prime-boost regime using DNA and recombinant vaccinia virus expressing the *Leishmania infantum* P36/LACK antigen protects BALB/c mice from cutaneous leishmaniasis," *Vaccine*, vol. 20, no. 7-8, pp. 1226–1231, 2002.

[202] E. Pérez-Jiménez, G. Kochan, M. M. Gherardi, and M. Esteban, "MVA-LACK as a safe and efficient vector for vaccination against leishmaniasis," *Microbes and Infection*, vol. 8, no. 3, pp. 810–822, 2006.

[203] E. A .F. Coelho, C. A. P. Tavares, F. A. A. Carvalho, et al., "Immune responses induced by the *Leishmania* (*Leishmania*) *donovani* A2 antigen, but not by the LACK antigen, are protective against experimental *Leishmania* (*Leishmania*) *amazonensis* infection," *Infection and Immunity*, vol. 71, no. 7, pp. 3988–3994, 2003.

[204] T. Okuno, M. Takeuchi, Y. Matsumoto, H. Otsuka, and Y. Matsumoto, "Pretreatment of *Leishmania* homologue of receptors for activated C kinase (LACK) promotes disease progression caused by *Leishmania amazonensis*," *Experimental Animals*, vol. 51, no. 4, pp. 335–341, 2002.

[205] E. F. Pinto, R. O. Pinheiro, A. Rayol, V. Larraga, and B. Rossi-Bergmann, "Intranasal vaccination against cutaneous leishmaniasis with a particulated leishmanial antigen or DNA encoding LACK," *Infection and Immunity*, vol. 72, no. 8, pp. 4521–4527, 2004.

[206] P. C. Melby, J. Yang, W. Zhao, L. E. Perez, and J. Cheng, "*Leishmania donovani* p36(LACK) DNA vaccine is highly immunogenic but not protective against experimental visceral leishmaniasis," *Infection and Immunity*, vol. 69, no. 8, pp. 4719–4725, 2001.

[207] E. A. Marques-da-Silva, E. A .F. Coelho, D. C. O. Gomes, et al., "Intramuscular immunization with p36(LACK) DNA vaccine induces IFN-γ production but does not protect BALB/c mice against *Leishmania chagasi* intravenous challenge," *Parasitology Research*, vol. 98, no. 1, pp. 67–74, 2005.

[208] M. J. Ramiro, J. J. Zárate, T. Hanke, et al., "Protection in dogs against visceral leishmaniasis caused by *Leishmania infantum* is achieved by immunization with a heterologous prime-boost regime using DNA and vaccinia recombinant vectors expressing LACK," *Vaccine*, vol. 21, no. 19-20, pp. 2474–2484, 2003.

[209] I. Ramos, A. Alonso, J. M. Marcen, et al., "Heterologous prime-boost vaccination with a non-replicative vaccinia recombinant vector expressing LACK confers protection against canine visceral leishmaniasis with a predominant Th1-specific immune response," *Vaccine*, vol. 26, no. 3, pp. 333–344, 2008.

[210] B. Dondji, E. Pérez-Jimenez, K. Goldsmith-Pestana, M. Esteban, and D. McMahon-Pratt, "Heterologous prime-boost vaccination with the LACK antigen protects against murine visceral leishmaniasis," *Infection and Immunity*, vol. 73, no. 8, pp. 5286–5289, 2005.

[211] W.-W. Zhang, H. Charest, E. Ghedin, and G. Matlashewski, "Identification and overexpression of the A2 amastigote-specific protein in *Leishmania donovani*," *Molecular and Biochemical Parasitology*, vol. 78, no. 1-2, pp. 79–90, 1996.

[212] D. R. A. Martins, S. M. B. Jeronimo, J. E. Donelson, and M. E. Wilson, "*Leishmania chagasi* T-cell antigens identified through a double library screen," *Infection and Immunity*, vol. 74, no. 12, pp. 6940–6948, 2006.

[213] E. Ghedin, W. W. Zhang, H. Charest, S. Sundar, R. T. Kenney, and G. Matlashewski, "Antibody response against a *Leishmania donovani* amastigote-stage-specific protein in patients with visceral leishmaniasis," *Clinical and Diagnostic Laboratory Immunology*, vol. 4, no. 5, pp. 530–535, 1997.

[214] F. A. A. Carvalho, H. Charest, C. A. P. Tavares, et al., "Diagnosis of American visceral leishmaniasis in humans and dogs using the recombinant *Leishmania donovani* A2 antigen," *Diagnostic Microbiology and Infectious Disease*, vol. 43, no. 4, pp. 289–295, 2002.

[215] A. Ghosh, S. Labrecque, and G. Matlashewski, "Protection against *Leishmania donovani* infection by DNA vaccination: increased DNA vaccination efficiency through inhibiting the cellular p53 response," *Vaccine*, vol. 19, no. 23-24, pp. 3169–3178, 2001.

[216] A. Ghosh, W. W. Zhang, and G. Matlashewski, "Immunization with A2 protein results in a mixed Th1/Th2 and a humoral response which protects mice against *Leishmania donovani* infections," *Vaccine*, vol. 20, no. 1-2, pp. 59–66, 2001.

[217] A. P. Fernandes, M. M. S. Costa, E. A. F. Coelho, et al., "Protective immunity against challenge with *Leishmania* (*Leishmania*) *chagasi* in beagle dogs vaccinated with recombinant A2 protein," *Vaccine*, vol. 26, no. 46, pp. 5888–5895, 2008.

[218] S. F. Guimarães Carvalho, E. M. Lemos, R. Corey, and R. Dietze, "Performance of recombinant K39 antigen in the diagnosis of Brazilian visceral leishmaniasis," *The American Journal of Tropical Medicine and Hygiene*, vol. 68, no. 3, pp. 321–324, 2003.

[219] F. J. Medrano, C. Cañavate, M. Leal, C. Rey, E. Lissen, and J. Alvar, "The role of serology in the diagnosis and prognosis of visceral leishmaniasis in patients coinfected with human immunodeficiency virus type- 1," *The American Journal of Tropical Medicine and Hygiene*, vol. 59, no. 1, pp. 155–162, 1998.

[220] S. Singh, V. Kumari, and N. Singh, "Predicting kala-azar disease manifestations in asymptomatic patients with latent *Leishmania donovani* infection by detection of antibody against recombinant K39 antigen," *Clinical and Diagnostic Laboratory Immunology*, vol. 9, no. 3, pp. 568–572, 2002.

[221] H. Veeken, K. Ritmeijer, J. Seaman, and R. Davidson, "Comparison of an rK39 dipstick rapid test with direct agglutination test and splenic aspiration for the diagnosis of kala-azar in Sudan," *Tropical Medicine & International Health*, vol. 8, no. 2, pp. 164–167, 2003.

[222] E. E. Zijlstra, Y. Nur, P. Desjeux, E. A. G. Khalil, A. M. El-Hassan, and J. Groen, "Diagnosing visceral leishmaniasis with the recombinant K39 strip test: experience from the Sudan," *Tropical Medicine & International Health*, vol. 6, no. 2, pp. 108–113, 2001.

[223] R. Porrozzi, M. V. Santos da Costa, A. Teva, et al., "Comparative evaluation of enzyme-linked immunosorbent assays based on crude and recombinant leishmanial antigens for serodiagnosis of symptomatic and asymptomatic *Leishmania infantum* visceral infections in dogs," *Clinical and Vaccine Immunology*, vol. 14, no. 5, pp. 544–548, 2007.

[224] S. O. Toz, K.-P. Chang, Y. Ozbel, and M. Z. Alkan, "Diagnostic value of RK39 dipstick in zoonotic visceral leishmaniasis in Turkey," *Journal of Parasitology*, vol. 90, no. 6, pp. 1484–1486, 2004.

[225] R. T. da Costa, J. C. França, W. Mayrink, E. Nascimento, O. Genaro, and A. Campos-Neto, "Standardization of a rapid immunochromatographic test with the recombinant antigens K39 and K26 for the diagnosis of canine visceral leishmaniasis," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 97, no. 6, pp. 678–682, 2003.

[226] D. Otranto, P. Paradies, M. Sasanelli, et al., "Recombinant K39 dipstick immunochromatographic test: a new tool for the serodiagnosis of canine leishmaniasis," *Journal of Veterinary Diagnostic Investigation*, vol. 17, no. 1, pp. 32–37, 2005.

[227] A. Dey, P. Sharma, N. S. Redhu, and S. Singh, "Kinesin motor domain of *Leishmania donovani* as a future vaccine candidate," *Clinical and Vaccine Immunology*, vol. 15, no. 5, pp. 836–842, 2008.

[228] Y. Belkaid, S. Mendez, R. Lira, N. Kadambi, G. Milon, and D. Sacks, "A natural model of *Leishmania major* infection reveals a prolonged "silent" phase of parasite amplification in the skin before the onset of lesion formation and immunity," *The Journal of Immunology*, vol. 165, no. 2, pp. 969–977, 2000.

[229] S. Méndez, S. Gurunathan, S. Kamhawi, et al., "The potency and durability of DNA- and protein-based vaccines against *Leishmania major* evaluated using low-dose, intradermal challenge," *The Journal of Immunology*, vol. 166, no. 8, pp. 5122–5128, 2001.

[230] R. T. Fujiwara, A. M. Vale, J. C. França da Silva, et al., "Immunogenicity in dogs of three recombinant antigens (TSA, LeIF and LmSTI1) potential vaccine candidates for canine visceral leishmaniasis," *Veterinary Research*, vol. 36, no. 5-6, pp. 827–838, 2005.

[231] Y. A. W. Skeiky, R. N. Coler, M. Brannon, et al., "Protective efficacy of a tandemly linked, multi-subunit recombinant leishmanial vaccine (Leish-111f) formulated in MPL® adjuvant," *Vaccine*, vol. 20, no. 27-28, pp. 3292–3303, 2002.

[232] R. N. Coler, Y. A. W. Skeiky, K. Bernards, et al., "Immunization with a polyprotein vaccine consisting of the T-cell antigens thiol-specific antioxidant, *Leishmania major* stress-inducible protein 1, and *Leishmania* elongation initiation factor protects against leishmaniasis," *Infection and Immunity*, vol. 70, no. 8, pp. 4215–4225, 2002.

[233] R. N. Coler, Y. Goto, L. Bogatzki, V. Raman, and S. G. Reed, "Leish-111f, a recombinant polyprotein vaccine that protects against visceral leishmaniasis by elicitation of $CD4^+$ T cells," *Infection and Immunity*, vol. 75, no. 9, pp. 4648–4654, 2007.

[234] A. Boarino, A. Scalone, L. Gradoni, et al., "Development of recombinant chimeric antigen expressing immunodominant B epitopes of *Leishmania infantum* for serodiagnosis of visceral leishmaniasis," *Clinical and Diagnostic Laboratory Immunology*, vol. 12, no. 5, pp. 647–653, 2005.

[235] M. Soto, J. M. Requena, L. Quijada, and C. Alonso, "Multicomponent chimeric antigen for serodiagnosis of canine visceral leishmaniasis," *Journal of Clinical Microbiology*, vol. 36, no. 1, pp. 58–63, 1998.

[236] N. Parody, M. Soto, J. M. Requena, and C. Alonso, "Adjuvant guided polarization of the immune humoral response against a protective multicomponent antigenic protein (Q) from *Leishmania infantum*. A CpG + Q mix protects Balb/c mice from infection," *Parasite Immunology*, vol. 26, no. 6-7, pp. 283–293, 2004.

[237] I. Molano, M. García Alonso, C. Mirón, et al., "A *Leishmania infantum* multi-component antigenic protein mixed with live BCG confers protection to dogs experimentally infected with *L. infantum*," *Veterinary Immunology and Immunopathology*, vol. 92, no. 1-2, pp. 1–13, 2003.

[238] K.-P. Chang and B. S. McGwire, "Molecular determinants and regulation of *Leishmania* virulence," *Kinetoplastid Biology and Disease*, vol. 1, article 1, pp. 1–7, 2002.

[239] K.-P. Chang, S. G. Reed, B. S. McGwire, and L. Soong, "*Leishmania* model for microbial virulence: the relevance of parasite multiplication and pathoantigenicity," *Acta Tropica*, vol. 85, no. 3, pp. 375–390, 2003.