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Review

Visceral leishmaniasis: An overview of vaccine adjuvants and their applications

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ABSTRACT

Although there has been an extensive research on vaccine development over the last decade and some vaccines have been commercialized for canine visceral leishmaniasis (CVL), but as yet no effective vaccine is available for anthroponotic VL which may partly be due to the absence of an appropriate adjuvant system. Vaccines alone yield poor immunity hence requiring an adjuvant which can boost the immunosuppressed state of VL infected individuals by eliciting adaptive immune responses to achieve required immunological enhancement. Recent studies have documented the continuous efforts that are being made in the field of adjuvants research in an attempt to render vaccines more effective. This review article focuses on adjuvants, particularly particulate and non-particulate ones, which have been assessed with VL vaccine candidates in several preclinical and clinical trials outlining the induction of immune responses obtained from these studies. Moreover, we have emphasized the applicability of multiple adjuvants combination for an improvement in the potential of a VL vaccine.

Contents

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1. Introduction

Visceral Leishmaniasis (VL), the most fatal form of leishmaniasis, caused by the protozoan parasite of *Leishmania (L.) donovani* complex is transmitted through the infected female Phlebotomine sandflies. Since the parasite migrates to reticuloendothelial cells of visceral organs (spleen, liver, bone marrow) hence the disease is named visceral leishmaniasis. VL is prevalent in the Indian subcontinent, East Africa where transmission is anthroponotic while in Europe, Latin America, North Africa, it is zoonotic and is responsible for half million cases each year. In India, it is most prevalent in Bihar, West Bengal, Uttar Pradesh, Jharkhand states and mainly affects poor communities residing in rural areas [1]. The key control measures rely primarily on chemotherapeutics regimens but have limited efficacy due to the side effects, toxicity, resistance, unaffordable prices, and low clinical success rate. VL is particularly characterized by progressive cellular immunosuppression and also it has been observed that most of the individuals residing in endemic regions remain asymptomatic acting as potent reservoirs. The emergence of Leishmania-HIV co-infection causes treatment failures and frequent relapses while Post Kala-Azar Dermal Leishmaniasis (PKDL), a sequel or complication of VL develops again due to the treatment failures [2–4]. Hence, there is a critical demand for an efficient vaccine development which can provide long-lasting protection in immunocompromised individuals. Moreover, cases of recovery from a primary infection accompanied by immunity to subsequent infections render vaccination a feasible leishmaniasis control method. However, despite a decade of immense efforts, to date, no candidate vaccine has been capable of providing the requisite level of protection against human VL often due to the lack of a suitable adjuvant. Besides, since VL is an infection of innate immune cells, so, targeting these cells with appropriate ligands as adjuvants could be a viable strategy for the disease containment [5,6]. Also, the innate immune cells are paramount in effectively modulating adaptive immune responses consequently unleashing the adjuvants' worth potential in generating modern vaccine combinations. In addition, traditional live attenuated or killed vaccines perhaps don't require adjuvant as these may inherently contain natural adjuvants because of their heterogeneous composition, but these vaccines cause non-specific reactogenicity [7]. To avoid this, protein-based vaccines come into existence which confer some advantages over classical ones, however these synthetic antigens are insufficiently immunogenic, thus necessitating adjuvants assistance in the rational vaccine design enhancing the efficacy of weak antigens by inducing robust immune responses that otherwise not significantly stimulated in the absence of adjuvant [8,9]. Further, the advancement of vaccine formulations including suitable adjuvants to licensing stages particularly in case of canine VL has provided an insight into the adjuvant role in fulfilling the unmet clinical needs. Therefore, selection of an appropriate adjuvant system containing immuno-stimulant or immunomodulator as well as its conjunction with vaccine candidates seemed to be essential for the success of any vaccine against VL. The addition of adjuvants is especially valued as it can also be employed in chemo-immunotherapy further leading to enhancement of the antigen immunogenicity and effectively modulating adequate immune responses further reducing the number of doses of anti-leishmanial agents required. Thus, vaccine development must take into account an inclusion of immunopotentiator or adjuvant which could improve the potency of immunogen by triggering a robust and long-lasting immunity.

2. Insight into cellular immunobiology of VL

In VL, *L. donovani* and *L. infantum* infection cause parasite establishment in invaded tissues like spleen, liver, bone marrow of human host thereby causing early accumulation of mononuclear phagocytic cells leading to hyperplasia of involved organs. Disease progression sustained due to the immunosuppressed state of the host; allowing uncontrolled division and dispersion of parasites leading to health deterioration or mortality. Recent studies have given new insights into the generation of various types of immune responses during *Leishmania* infection which evokes different immune cells resulting in the development of antileishmanial responses. VL was initially thought to be associated to the dominant Th2 type of immunity wherein IL-4 and IL-13 production result into disease progression while Th1 immune response, characterized by IFN-γ and TNF-α secretion, is responsible for infection control. However, in recent years, it became evident that this Th1/Th2 dichotomy is not strictly observed in humans though there is a participation of other immune cell types yielding more complicated and mixed immunity. In several studies, VL patients express elevated level of IFN-γ along with regulatory cytokines IL-10 and IL-6 and minimal level of TNF-α, IL-2, and IL-4 in both mRNA transcript and serum in lesional tissues during the acute phase which suggested that the disease pathogenesis can't be elucidated merely by immune tolerance or Th2 polarization [10]. Moreover, human VL is also linked with the elevated level of IL-10 which contributes to effector T-cell deficiency [11] and as per another subsequent finding, IL-10 neutralization in ex-vivo cell assays promoted significant parasite clearance in splenic aspirates of active VL patients, thus suggesting the critical role of IL-10 as a key suppressor of leishmanicidal immune mechanism [12].

Furthermore, another Th subset i.e., Th17 cells (proinflammatory CD4 + T cells) was reported to play a protective role during VL. In agreement to this, a study by Pitta et al. in 2009 demonstrated the protective role of Th17 cells in human VL wherein IL17 and IL22 along with Th1 cytokines were strongly associated with immunoprotection against the disease [13]. Likewise, in another investigation, the synergistic activity of IL-17A with IFN-γ to potentiate NO production against *L. infantum* challenge was reported [14]. It has been well demonstrated that Th17 cells are centrally regulated by IL-27 cytokine by blocking Th17 expansion during VL [15]. Increased mRNA expression of IL-27 and IL-21 along with elevated levels of circulating IL-27 was reported by Ansari et al. in 2011 [16]. Moreover, the role of IL-21 in immunosuppression by inducing IL-10 cytokine [17] was implicated and thus, these studies supported the fact that IL-27, as well as IL-21, can potentially inhibit the protective Th17 lineage thus facilitating parasite propagation thereby disease progression.

Recent studies have documented the implication of regulatory T cells (Treg) in the suppression of effector T-cell functions. Nylen et al. demonstrated that in VL patients, CD4 + CD25 − Foxp3− or adaptive Treg cells were found to be involved in increased mRNA expression of IL-10 with almost no accumulation of CD4 + CD25 + Foxp3+ (natural Treg cells), however, conversely, Rai et al. in 2012 reported the involvement of Foxp3 + T-cells in inhibiting T-cell activation by producing IL-10 thereby suppressing antileishmanial immunity [18]. Thus, the definitive role of Treg cells in the context of human VL is yet to be demonstrated.

Another lineage, CD8 + T-cells are capable of directly acting on infected target cells but its contribution in disease progression or pathology or cure is still controversial and more often depends on the infection model, although its role has been demonstrated in protection against *L. donovani* infection in cured VL individuals [19]. However, Gautam et al. described CD8 + T cells as exhausted phenotype characterized by high expression of CTLA-4, PD-1, along with IL-10 with limited protective ability [20]. Since very few reports are available on the functions of CD8 + T cells in human VL, so an extensive investigation is required in order to elucidate the anti-Leishmanial potential of these cells.

In spite of the fact that B-cells function in disease exacerbation in the case of *L. donovani* infection, the roles of B-lymphocytes and antibodies have not been considered of much significance in disease pathogenesis [21]. Although, elevated levels of anti-leishmanial IgG and its subtypes were reported in active VL patients [22,23] and moreover, antibodies have been useful in VL diagnosis sometimes, but still, these can't predict disease outcome.

Clearly, a better understanding of the precise mechanism of different cytokines involved in VL as well as immune pathways being operated during this disease is required to design a suitable vaccine that can effectively modulate the immune system towards protection or cure.

3. Vaccine candidates for VL

A large number of vaccination strategies have been employed against VL, but as yet there is no effective human vaccine available in the market; though, some of them are in the pipeline. Development of *Leishmania* vaccine has proven to be a complicated task due to insufficient knowledge of parasite pathogenesis and intricacy of immune responses required for protection [24]. First generation vaccine includes whole parasite based vaccine which may be either killed or attenuated. Leishmanization is the inoculation of live virulent *L. major* promastigotes and was practiced for several years in many countries but it is now abandoned due to the safety issues. Whole killed parasite based vaccines were also tested dated back to 1940s and reached to clinical trials. Likewise, several attenuated parasites have been utilized for prophylactic studies but concerns regarding their reversion to virulent forms make them inappropriate for human use. Some examples of genetically modified parasites lacking crucial genes are biopterin reductase, cysteine proteases, centrin which has been shown to confer protection against virulent parasite strains [25–27]. Most of the vaccination studies have been concentrated on second-generation vaccines including recombinant proteins. Several different antigens that have been tried in various animal models are *L. infantum* heat shock protein (HSP)-70, paraflagellar rod protein (PFR)-2, kinetoplastid membrane protein-11 (KMP-11) [28], amastigote specific protein A2 [29], sterol 24-c-methyltransferase (SMT) [30], hydrophilic acylated surface protein B1 (HASPB) [31], Leishmanial antigen ORFF [32]. Nevertheless a few of them advanced to clinical trials; for instance, a polyprotein recombinant vaccine Leish 111f comprising of thiol-specific antioxidant (TSA), *L. major* stress-inducible protein-1 (LmSTI1) and *Leishmania* elongation initiation factor (LeIF), formulated with Monophosphoryl Lipid-Stable Emulsion adjuvant, has been shown to confer protection in VL rodent models [33] which also progressed to human clinical trials [34]. Subsequently, Leish-F3 (composed of Nucleoside Hydrolase from *L. donovani* and SMT from *L. infantum* formulated with glucopyranosyl lipid A-Stable Emulsion) and Leish-F3+ (Cysteine Protease B added to Leish-F3) also moved to phase I clinical trials [35,36]. Apart from these, third generation vaccines comprised of DNA vaccines like *Leishmania* homologue of mammalian RACKs (LACK) [37], NH36 [38], ORFF [39], Cysteine proteinases [40], KMP11 [41]. Despite enormous progress being made in vaccines development against murine VL, development of a vaccine against human VL has been difficult so far thus demanding new vaccination strategies involving an adequate adjuvant system for generating long-term immunity.

4. Different adjuvants tested for candidate vaccines against VL

Adjuvants are molecules that augment the potency of a specific humoral and cellular immune response against inoculated antigens, causing least toxicity or long-lasting immune effects on their own. In order to improve vaccine efficacy and safety, adjuvants must be adapted depending on the nature of the antigen, immunization schedule, administration route, type and duration of required immunity and pharmaceutical parameters [8,42]. There are several potential benefits of employing immunologic adjuvants for candidate vaccines against VL such as enhancing immunogenicity and potency, modulating T cell phenotype to Th1 type, reducing the number of immunization doses, prolonged induction of specific effector CD4 + and CD8 + T-cells and extending magnitude of neutralizing antibody responses [9].

Since recombinant protein vaccines are often poorly immunogenic conferring weak immune response thereby necessitating the requirement of adjuvant that could enhance the immunogenicity of vaccines switching towards protective immune response following the natural course of infection. However, in most of the cases, the host is already infected with the pathogen and is immunosuppressed, so therapeutic vaccine is needed and in this case, adjuvants may have to promote immunity by boosting up immunosuppressed state in infected hosts. Recent research has shed considerable light on the adjuvant biology and mechanistic approaches leading to its wide applications in different generations vaccines.

Adjuvants which were used for VL till date can be broadly classified into 2 major categories on the basis of their mode of actions:

(1) Non-particulate or immunostimulatory adjuvants directly act on the immune system to increase responses to antigens and are mostly pathogen-associated molecular patterns (PAMPs) which specifically bind to toll-like receptors (TLRs) or nucleotide-binding oligomerization domain-like receptors (NLRs). The immune system recognizes PAMPs and the endogenous receptors bind microbial ligands to trigger different types of immune responses [42]. E.g. monophosphoryl lipid A (MPL-A), muramyl di- or tripeptides and derivatives (MDP/MTP-PE), BCG, saponins, cytokines, or CpG oligonucleotides.

(2) Particulate adjuvants enhance the specific immune response to the antigen by targeting antigens specifically to the site of action and modulating the immune responses in an optimal manner, including controlled release and depot delivery systems. They also enhance and facilitate the absorption, uptake, and cross-presentation of antigens in APCs. Moreover, the particle delivery system has the potential for successfully delivering *Leishmania* antigens along with additional immunostimulatory adjuvants in or onto the particle carrier system. E.g. mineral salts, liposomes, or polymer-based delivery systems [8].

5. Non-particulate adjuvants

5.1. Bacillus calmette guerin (BCG)

BCG, an attenuated form of *Mycobacterium bovis,* has been a widely acceptable adjuvant in human use and induces Th1 type of immunity associated with stimulation of TLRs and IL-12 production. In a longitudinal study carried out in Sudan, autoclaved *L. major* (ALM) plus BCG combination elicited remarkably high IFN-γ in 76.9% of vaccinated individuals and also increases survival period of infected hamsters [43,44]. Further, in 2008 Ferreira et al. reported significant protection against *L. chagasi* challenge characterized by a predominant Th1 response in mice vaccinated with recombinant cysteine proteinase/BCG [45]. Later studies also demonstrated protective efficacy of *L. donovani* high molecular weight antigenic fractions in combination with BCG in hamsters [46]. Subsequently, Kumari et al. reported that Th1 stimulatory polyprotein of soluble *L. donovani* promastigotes ranging from 89.9 to 97.1 kDa exhibited long-lasting protection against *L. donovani* challenge [47]. Based on this result some recombinant proteins identified from this fraction were developed as recombinant ones and were found to

Table 1
List of non-particulate adjuvants that have been tested with vaccine candidates against VL.

Adjuvants with mode of action	Vaccines/Routes of administration	Against infection	Test models	Remarks [References]
BCG-Activates phagocytosis and induces Th1 biased immune response	Autoclaved *L. major*/i.d.	*L. donovani*	Sudanese volunteers	76.9% immunized individuals produce high IFN-γ, DTH response in 61.54% individual[43]
	Autoclaved *L. major* (ALM) or *L. donovani* (ALD)/i.d	*L. donovani*	Hamsters	94.3% and 86.1% parasite reduction in ALM + BCG and ALD + BCG resp., ↑ mean survival period [44]
	L. chagasi Cysteine proteases/s.c.	*L. chagasi*	BALB/c	100 fold parasite reduction in spleen, significant secretion of NO and IFN-γ [45]
	L. donovani whole protein fractions (134–64.2 kDa)/i.d.	*L. donovani*	Hamsters	Fraction E (60–70 kDa) exerted 73.7% and 80.2% parasite inhibition in spleen and liver resp. and *in vitro* stimulatory responses and NO induction, ↑ +Leishmania-specific IgG levels [46]
	L. donovani soluble prom-fractions and subfractions/i.d.	*L. donovani*	Hamsters	~90% parasite inhibition, ↑ LTT response, IFN-γ, IL-12, DTH response, Leishmania-specific IgG2 [47]
	L. donovani Th1 stimulatory proteins-p45, rLdTPR, rLdEno, rLdAld, rLdSir2RP, rLdcTryP, rLdTCP20, rLdAdoHcy, rLdiPGAM/i.d.	*L. donovani*	Hamsters	Prophylactic efficacy (~60–90%), ↑ iNOS, mRNA transcript and IFN-γ, IL-12, IL-12p40 and TNF-α, IgG2 and ↓ IL-4, IL-10, and TGF-β [48–54]
P. acnes-Induces Th1 biased immune response	*L. donovani* rA2/i.p.	*L. donovani*	BALB/c mice	89% LDU reduction in liver, strong anti-A2 Ab response, splenocyte proliferation, ↑ IFN-γ, IgG1, IgG2a, IgG2b, IgG3 [29]
	L. infantum chagasi rLdccys1/s.c (Immunotherapy)	*L. chagasi*	Naturally infected Dogs	7 log reduction in splenic parasite burden, ↑ DTH response, IFN-γ, IgG2, ↓ IgG1, IL-10 [56]
MDP-Stimulates cellular and cytokine responses, eliciting Ab production	*L. infantum* LiESAp/s.c.	*L. infantum*	Beagle Dogs	100% protection, anti-LiESAp IgG2 reactivity, +LiESAp specific LTT response, ↑ IFN-γ, enhanced NO-mediated anti-leishmanial activity of canine macrophages, 92% vaccine efficacy in field trial [60]
IL-12-Stimulates proliferation and release of IFN-γ from T-cell and NK cells generating cellular immune responses	*L. donovani* rHASPB1/s.c.	*L. donovani*	BALB/c mice	78% parasite reduction in liver, induced IL-12p70, IFN-γ producing CD8 + cell, ↑ IgG1 response [31]
	L. infantum Cysteine Peptidases rCPA and rCPB/s.c.	*L. infantum*	Beagle Dogs	81% dogs parasite positive (no protection), ↑ IFN-γ, comparable IL-4, antigen specific Ab detected [63]
	L. donovani Serine proteases/s.c.	*L. donovani*	BALB/c mice	91 and 89% parasite reduction in spleen and liver, ↑ DTH, Splenocyte proliferation, IFN-γ, TNF-α, IgG2 and ↓ IL-10, IL-4, IgG1 [62]
MPL-A-Induces strong humoral and T-cell response through TLR-4 signaling cascade	mix of TSA, LeIF, LmSTI1/s.c.	*L. chagasi*	Dogs	Produced specific IgG against individual vaccine components, generated a strong immunological memory, IgG2/lgG1 [70]
	SMT/s.c.	*L. infantum*	C57BL/6 mice	55 and 117 fold parasite reduction in liver and spleen resp., ↑induction of Ag-specific CD4 + cells producing IFN-γ and TNF-α, IL-2 and CD8 + cells producing TNF-α and IFN-γ [30]
	Leish111f/i.m., s.c.	*L. infantum*	BALB/c, C57BL/6, C57BL/10 mice, LVG golden Syrian Hamsters	91.7% and 99.6% parasite reduction in spleen in mice and hamster resp., ↑ CD4 + cells producing IFN-γ, TNF, IL-2 and ↓ CD8 + cells, IL-4 [33]
	Leish111f/s.c.		Humans	Safe and immunogenic, induced T-cell production of IFN-γ [34]
	Leish110f, Glucantime/s.c. (immunochemotherapy)	*L. chagasi*	Mongrel Dogs	75% Survival probability, high cell proliferative response, reduction of specific Ab titre [75]
	rF14/i.m.	*L. donovani*	Hamsters	46% and 36% parasite reduction in spleen and liver resp.,100% survival, ↑ DTH, LTT, IgG, IL-12p40, NO, ↓ IL-10 [69]
	KSAC (KMP11, SMT, A2, and CPB)/s.c.	*L. infantum*	C57BL/6 mice	93% and 66% parasite reduction in liver and spleen resp., induction of antigen-specific CD4 T cells producing IFN-γ, TNF-α, IL-2 [73]
	78kD, SSG, Cisplatin/s.c. (Immunochemotherapy)	*L. donovani*	BALB/c mice	84.3–99.4% parasite reduction in liver and spleen, ↑ DTH responses, IgG2a, IFN-γ and IL-2, ↓ IgG1, IL-10, IL-4 [76]
	L. braziliensis antigen/s.c.	*L. infantum*	Dogs	127.5 and 7 times parasite reduction in skin and bone marrow, ↑ CD14 + monocytes, NK cells, TCD4 + IFN-γ and TCD8 + IFN-γ, TNF-α, ↑ CD21 + B cells, ↓ TCD4 + IL-4, TCD8 + IL-4, IL-10 [74]
GLA-SE-Induces strong humoral and T-cell response through TLR-4 signaling cascade.	Leishf3/i.v.	*L. donovani, infantum*	C57BL/6 and BALB/c mice	Significant reductions in parasite burden in liver, ↑ IFN-γ, TNF and IL-2, ↓ IL-5 and IL-10 [35]
	79kD Chimeric fusion protein [8E (*L. braziliensis*), p21 (*L. major*) and SMT (*L. infantum*)]/s.c.	*L. donovani*	C57BL/6 mice	Exhibited lower parasite burden, ↑ antigen-specific IgG1, and IgG2a, IFN-γ, ↓ IL-5 [79]

Table 1 (*continued*)

Adjuvants with mode of action	Vaccines/Routes of administration	Against infection	Test models	Remarks [References]
CpG ODN-Boosts humoral and cellular responses promoting Th1 type of immunity through TLR-9 signaling mechanism.	*L.* rORFF and *L. donovani* Soluble Antigen/i.m.	*L. donovani*	BALB/c mice	60–80% parasite reduction in liver, ↑ IFN-γ, IL-12, IgG2a, NO production [80,81]
	L. donovani Gp63/i.m., s.c.	*L. donovani*	BALB/c mice	10^7 and 10^{10} fold parasite reduction in liver and spleen resp., ↑ DTH response, NO, IgG2a/IgG1, IFN-γ, IL-12, ↓ IL-4, IL-10 $^{\text{fold and } 101}$ [82]
	L major ribosomal proteins L3 (LmL3) and L5 (LmL5)/s.c.	*L. chagasi*	BALB/c mice	1.5–2 log reduction in parasite burden in liver, spleen, lymph node, ↑ IFN-γ, ↓ IL-4 and IL-10 [83]
Saponin-Increases anti-*Leishmania* IgG isotypes, together with higher levels of lymphocytes, particularly circulating CD8 + T-lymphocytes	FML/i.p., s.c	*L. donovani*	BALB/c mice, Swiss Albino	84–85% parasite reduction in liver, 79% increase in proliferative response, 80–89% increase in Ab response, ↑ IgG2a [109]
	L. donovani glycoprotein (Gp36)/s.c	*L. donovani*	BALB/c mice	68.1% parasite reduction in liver, 82.6% increase in IgG2a, proliferative response (53.5%), DTH response (37.8%) [110]
	FML/s.c.	*L. donovani*	Dogs	95% protection, + DTH response, Anti-FML ↑ humoral response [92]
	FML/s.c. (Immunotherapy)	*L. donovani*	BALB/c mice, Mongrel Dogs	90–94.7% reduction in liver parasitic load, ↑ DTH, IgM, IgG1, IgG2a, IgG2b, ↓ IL-10 [87,91]
	FML/s.c.	*L. chagasi*	Swiss Albino	95% and 86% parasite reduction in liver in QS21-FML and deacylsaponins-FML groups resp., ↑ IgG, IgG1, IgG2a, IgG2b, IgG3 in QS21-FML vaccinated animals, DTH, IFN-γ, CD4 + T in spleen [89]
	Leishmune/s.c. (Immunotherapy)	*L. chagasi*	Mongrel Dogs	↑ Anti-FML IgG2, 75% positive DTH response [93]
	rA2, Leishtec/s.c.	*L. chagasi, infantum*	Dogs	71% asymptomatic, ↑ IFN-γ, anti A2 IgG, IgG2, ↓ IL-10 [104,105]
	L. infantum Ribosomal proteins/s.c.	*L. chagasi*	BALB/c mice	4.7 and 9.3 log parasite reduction in liver and spleen resp., IL-12 dependent production of IFN-γ by CD4 + and CD8 + T cells, +NO,↓ IL-4 and IL-10 [111]
	L. donovani NH36/s.c.	*L. chagasi*	BALB/c mice	90.5–88.23% parasite reduction, ↑ DTH response, TNF-α/IL-10 CD4 + producing cells, IgM, IgG1, IgG2a, IgG2b, IFN-γ/IL-10 producing CD4 + and CD8 + T cell [112]
	L. donovani NH36 and F3/s.c.	*L. chagasi*	C57BL/6	95% and 87% parasite reduction in spleen resp., 49% and 39% reduction in splenomegaly resp., 36% and 26% prevention in increase in total DC count resp. [113]
	rLiHyp1, rLiHyp6, rLiHyT, rLiHyV, rLiHyS, rLiHyD, *L. braziliensis* proteins, rEnolase, rSGT, rSMP-3/s.c.	*L. infantum*	BALB/c mice	Significant parasite reduction in liver, spleen, bone marrow, lymph nodes, ↑ IFN-γ, IL-12, GM-CSF, IgG2a, ↓ IL-4 and IL-10 [95–103]
	LbSapSal/s.c.	*L. chagasi*	Dogs	69–78.9% parasitic reduction in spleen, expansion of CD21 + B cells, CD4+, CD8 + T cells, ↑ NO, LTT response, IgG, IgG1, IgG2, TNF-α, IFN-γ, IL-12, ↓ IL-4, TGF-β [106–108]

Footnotes: ↑ – Increase, ↓ – Decrease, resp. – respectively, Ab – antibody, i.d. – intradermal, s.c. – subcutaneous, i.m. – intramuscular, i.p. – intraperitoneal, DTH – Delayed Type Hypersensitivity, LTT – Lymphocyte Transformation Test, NO – Nitric Oxide, LDU – Leishman Donovan Unit, PBMCs – Peripheral Blood Mononuclear Cells.

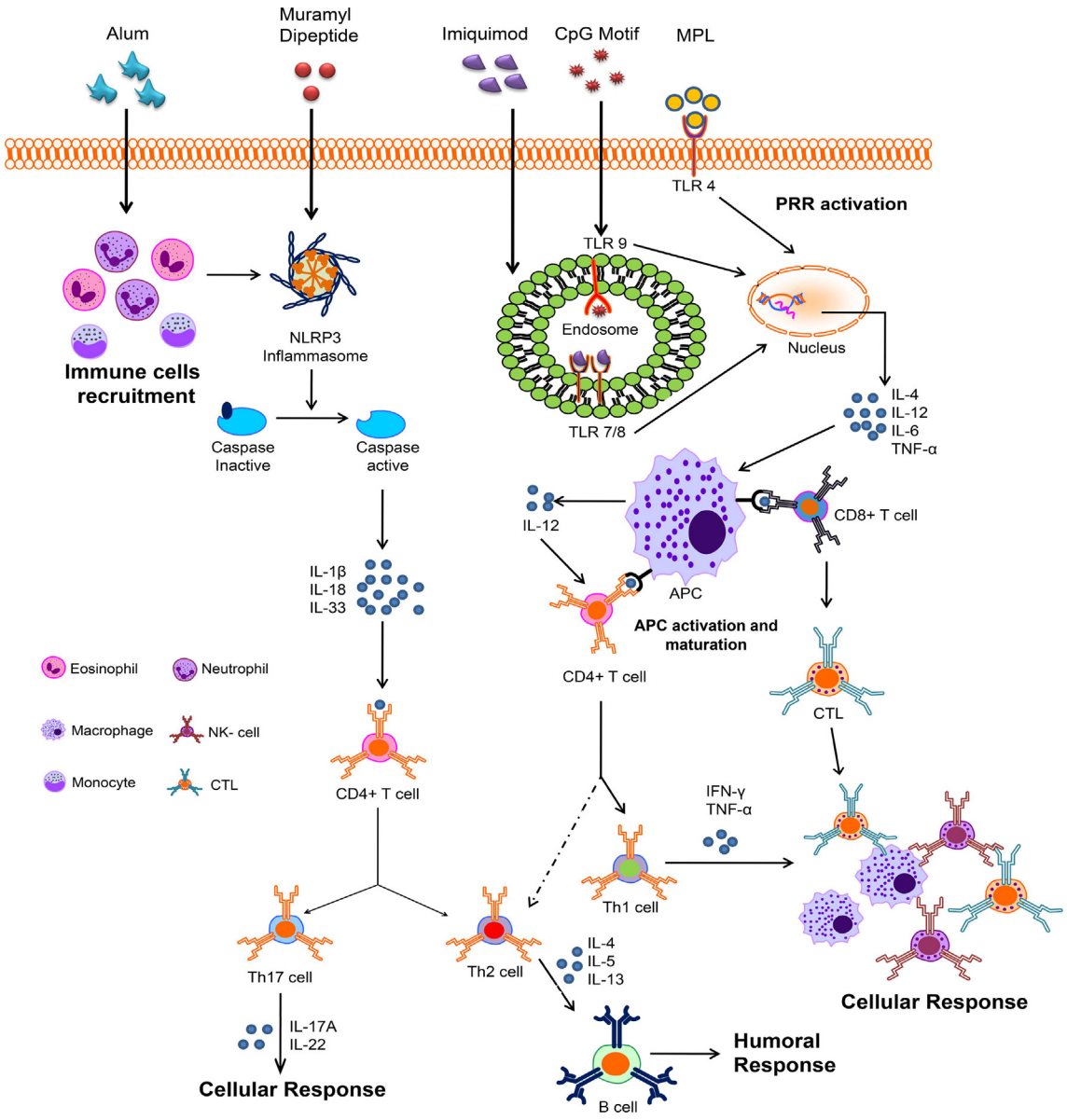

Fig. 1. Mechanisms of actions of adjuvants. Adjuvants may act through one or multiple synergistic signaling mechanisms to elicit immune responses. Alum adjuvant leads to rapid recruitment of polymorphonuclear immune cells at the site of vaccination creating an inflammatory environment. Alum and muramyl dipeptide (MDP) act through inflammasome activation and caspase-1 dependent secretion of cytokines IL-1β, IL-18, and IL-33 which further promotes CD4 + T cell differentiation to Th2 and Th17 cells augmenting antibody production and cellular response development respectively. However, the ultimate fate of Th17 cells is yet to be resolved in the VL scenario. TLR agonists (MPL, CpG Motif, Imiquimod) adjuvants recognize various pathogen recognition receptors (PRR) that activate transcription factors inducing the production of key cytokines to prime naive CD4 + T-cells towards the specific Th1 phenotype and also enhance antigen presentation to CD8 + T-cells generating cellular response against VL infection.

be offering considerable prophylactic efficacy against experimental VL in Syrian hamsters [48–54] (Table 1).

5.2. Bacteria and derivatives

The inclusion of micro-organisms and their metabolic products into several vaccines has been shown to augment immune responses acting as adjuvants. *Propionibacterium acnes* (*P. acnes*) is a Gram-positive bacterium associated with specific Th1 skewed immune response characterized by synthesis of various pro-inflammatory cytokines primarily IL-12 and IL-18 thereby stimulating IFN-γ production [55]. In a study, *L. donovani* amastigotes specific recombinant A2 protein with *P. acnes* conferred significant protection, however, yielding mixed Th1/Th2 phenotype [29]. Moreover, immunization with recombinant cysteine proteinase (rLdccys) adjuvanted with *P. acnes* elicited protective immunity in murine model against *L. chagasi* infection and also generated significant immunotherapeutic effects in dogs against CVL [45,56].

Another bacterial derivative, muramyl dipeptide (MDP) is a synthetic immunoreactive peptide inducing an immune response by generating IFN-γ and other cytokines further stimulating lymphocytes differentiation and proliferation (Fig. 1) [8,57]. In a study, a formulation of excretion/secretion antigen purified from culture supernatant of *L. infantum* promastigotes (LiESAp) with MDP possesses prophylactic as well as therapeutic activity in *L. infantum* infected dogs [58,59]. It also confers protection in naturally exposed dogs of endemic regions of South of France [60] (Table 1).

5.3. Cytokines

The adjuvant potential of different cytokines including IL-12, Granulocyte-monocyte colony stimulating factor (GM-CSF), IFN-γ

has been evaluated for vaccines against VL. IL-12 is a recently characterized immunomodulatory cytokine stimulating Th progenitor cell differentiation into Th1 subset as well as activation of humoral responses to both T-dependent and T-independent antigens. It is a potent inducer of CD8 T-cells, NK cells, T-cell proliferation and IFN-γ secretion [61]. Hence, the use of *L. donovani* secretory serine proteases (pSP) with IL-12 exhibited sustained protection through activating Th1 cytokines mediated pathway [62]. Additionally, *L. donovani* HASPB1 combined with murine rIL-12 also conferred protection against hepatic and splenic infection following *L. donovani* challenge along with the development of adaptive immune response [31]. However, preparation of canine IL-12 with cysteine peptidases doesn't protect dogs from *L. infantum challenge* [63] (Table 1. The cytokine recombinant GM-CSF has been reported to be involved in reversing neutropenia and reducing secondary infections in VL patients [64]. However, the use of GM-CSF as an immunomodulator was subsequently investigated as a treatment for mucosal infections [65]. Another cytokine IFN-γ has been employed as an adjunct in immunochemotherapy with pentavalent antimony [66].

5.4. TLR agonists

Toll-like receptors (TLRs) are a group of pathogen-associated molecular patterns (PAMPs) recognition receptors that play a crucial role in modulating host immune system. TLRs 1, 2, 4–6 are expressed on cell membrane surface while TLR 3, 7–9 are expressed on endosomes. Various preclinical and clinical studies have suggested that purified TLR agonists could induce an adaptive immune response and further be employed as potential vaccine adjuvants. Of all the TLR agonists, TLR-4, 7 and 9 agonists have been exploited as immune-enhancers for candidate vaccines against VL.

(A) TLR-4 agonists: Lipid A derivatives

Lipid A mimetic Monophosphoryl lipid-A (MPL-A), a TLR4 agonist has immunomodulatory potential and is safe and efficacious in inducing an immune response to a range of protein vaccines. MPL-A is mucosally administered leading to systemic immune response and also promotes Th1 type of response (Fig. 1). Of all the TLR agonists, MPL is the only licensed, non-toxic adjuvant commercially available today against various infectious diseases [5]. Various studies have demonstrated that MPL-A inclusion in the antigenic preparation induces Th1 profile with significant parasitic burden reduction [67–69]. MPL-Stable Emulsion (MPL-SE) with squalene oil, a modified form with improved efficacy has been co-administered with recombinant isolates (LeIF, LmSTI, and TSA) of Leish111f in the cocktail which generated a strong immunological memory and Th1 type of immunity against canine VL [70]. Interestingly, immunization with Leish111f (Leishf1) adjuvanted with MPL-SE demonstrated a strong humoral and T cell responses in rodent models [33] and was also safe and immunogenic in endemic contacts of the Indian population in phase I trial [34]. However, in another report, Leish111f-MPL-SE failed to prevent disease progression and protection of beagle dogs in phase III trial [71] but provided clinical cure in many dogs with VL thus depicting potential immunotherapeutic efficacy of the vaccine [72]. Furthermore, rSMT immunization with MPL-SE in murine model leads to the induction of CD4 + and CD8 + cells expressing multiple Th1 cytokines [30]. In another immunization protocol, co-administration of KSAC (fusion of KMP11, SMT, A2, and CPB) with MPL-SE protected mice from *L. infantum* infection [73]. Moreover, immunotherapy with *L. braziliensis* antigens with MPL (LBMPL) vaccine resulted in immune response polarization with a significant enhancement of clinical and immune status and parasite burden reduction along with potential to block vector transmission [74]. Further investigation puts an insight into the use of MPL-A as immunochemotherapy with drugs N-methyl meglumine antimoniate, cisplatin and sodium stibogluconate and responsible for higher survival probability and disease resolution thus proving an alternative for VL treatment [75–77] (Table 1).

Another lipid A derivative, Glucopyranosyl lipid stable emulsion (GLA-SE), a modified form of MPL adjuvant induces APCs activation and IFN-γ production by antigen-specific T cells through TLR-4 signaling, thus suggesting multifunctional immunomodulatory activities [5,78]. In a study, co-administration of GLA-SE with Leishf3 enhanced the antigen immunogenicity inducing an antigen-specific humoral and robust T-cell immune response in mice as well as healthy volunteers in the United States [35]. Further, a 79 kDa chimeric protein comprising of 8E, p21, and SMT in formulation with GLA-SE also elicited antigen-specific protective Th1 response [79] (Table 1).

5.5. TLR-9 agonist: CpG-Oligodeoxynucleotide (CpG-ODN)

The adjuvant effect of CpG-ODN is mediated through stimulating antigen presenting cells (APCs) to produce various cytokines through TLR-9 signaling, inducing the coordinated production of IL-6, IFN-γ and IL-12 by NK cells, B-, and CD4 + T lymphocytes respectively (Fig. 1) and also enhance antigen presentation to CD8 + T lymphocytes [5] thus making CpG-ODN a potent adjuvant for vaccine. The inclusion of CpG-ODN with rORFF and soluble Leishmania antigen (SLA) resulted in strong Th1 promoting isotype with enhanced IFN-γ and IL-12 secretion and parasite burden reduction [80,81]. Moreover, heterologous prime-DNA/boost-protein immunization regime using gp63 in presence of CpG also showed a polarized Th1 response fold and 10^1 [82]. CpG-ODN adjuvanticity was also evaluated with *L. major* ribosomal proteins based vaccines inducing protection against *L. chagasi* [83]. Further, it was reported that mannosylated liposomal CpG-ODN more effectively leads to complete parasite elimination than liposomal or free CpG-ODN [84]. However, it has now been shown that liposomal formulation of CpG-ODN combination with a sub-curative dose of a drug miltefosine enhances the anti-leishmanial activity yielding ~97% inhibition of parasite multiplication in both mice and hamsters [85] (Table 1).

5.6. Saponin

Saponins are plant-based natural glycosides of steroid or triterpene having the capacity to trigger mammalian immune system through stimulating both Th1 immunity and the production of cytotoxic T-lymphocytes (CTLs) thus making it a potential adjuvant for various vaccines in preclinical and clinical studies [86]. Saponin and its modified forms have been used in combination with Fucose Mannose Ligand (FML) both in prophylactic and therapeutic vaccination regimens eliciting a prominent reduction in parasitic burden and signs of disease along with specific immunity induction in rodent models [87–91]. FML antigen in formulation with Quil A saponin has been also tested against CVL and is now commercialized under the name of Leishmune in Brazil which confers strong and long-lasting protection [92] with effective therapeutic efficacy in infected dogs [87]. Furthermore, in another study, vaccination with Leishmune vaccine in infected mongrel dogs modulate their potential infectiosity to the vector with an interruption in disease transmission [93]. Encouraging with results, the effect of Leishmune was also observed in healthy seronegative dogs during a 2-year trial which showed strong immunogenicity induction [94]. Besides these, saponin has been also included as an adjuvant with other recombinant hypothetical proteins in various prophylactic studies [95–103]. Moreover, immunization with rA2 antigen plus

Table 2
List of particulate adjuvants that have been tested with vaccine candidates against VL.

Adjuvants with mode of action	Vaccines/routes of administration	Against infection	Test models	Remarks [References]
Liposome-Able to encapsulate and deliver antigens to specific immune cells along with humoral and T-cell proliferative responses	*L. donovani* Promastigote membrane Antigen (L Ag)/i.p	*L. donovani*	BALB/c mice	57% and 59.4% protection in liver and spleen resp., ↑ DTH response, splenocytes proliferation, leishmanicidal activity, IgG2a and IgG2b [116]
	L. donovani promastigote soluble Ag in non-PC liposome (EPC-sLAg) and Escheriosome (EL-sLAg)/i.p.	*L. donovani*	Hamsters, BALB/c mice	EPC-sLAg-38% parasite reduction in spleen, 14.8% increase in CD4 + T-cells, ↑ IFN-γ, IL-4 EL-sLAg-84% parasite reduction in spleen, 23% and 22% increase in CD4 + and CD8 + T-cells, ↑ LTT response, NO, IFN-γ, IL-4 [118]
	Non-coding plasmid DNA and soluble *L.* antigen/i.p.	*L. donovani*	BALB/c mice	10^{9}–10^{11} fold parasite burden reduction in liver and spleen, ↑ DTH response, IgG, IgG2a, IgG2a/IgG1 ratio, IFN-γ, IL-12, ↓ IL-4, IL-10 [119]
	L. donovani rGP63/s.c.	*L. donovani*	BALB/c mice	86% and 81% resistance to hepatic and splenic infection resp., 100% survival, ↑ DTH response, NO, IgG2a, IFN-γ, IL-12, CD4 + and CD8 + IFN-γ producing T cells, ↓ CD4 + IL-4 producing T cells [120]
Montanide-Induces both Th1 type cellular and humoral immune response	*L. infantum* rHASPB1 and rH1/i.d.	*L. infantum*	Dogs	50% asymptomatic, ↑ anti-HASPBI antibody titre[122]
Freund's Adjuvants-Prolongs antigen persistence, activates phagocytosis, enhances co-stimulatory signals and Ab production	*L. chagasi* rLcr1 and *L. chagasi* amas 30kD/s.c., i.p.	*L. chagasi*	BALB/c, C3H.HeJmice	Partial protection, detection of IL-2, IL-10 and IFN-γ in supernatants from lymphocytes stimulated by antigen [125,126]
	L. rORFF and rBT1/s.c.	*L. donovani*	BALB/c mice	10–81% parasite reduction in liver, splenocyte proliferation, detectable antigen specific Ab titre [127]
	L. donovani ribosomal protein (rLdP1)/s.c.	*L. donovani*	Hamster	66.6% survival, low lymphocytes proliferation, unable to stimulate the release of Th1 type cytokines significantly [128]

Footnotes: ↑ – Increase, ↓ – Decrease, resp. – respectively, Ab – antibody, i.d. – intradermal, s.c. – subcutaneous, i.m. – intramuscular, i.p. – intraperitoneal, DTH – Delayed Type Hypersensitivity, LTT – Lymphocyte Transformation Test, NO – Nitric Oxide, LDU – Leishman Donovan Unit, PBMCs – Peripheral Blood Mononuclear Cells.

saponin, induced partial protection in beagle dogs against *Leishmania* infection and is currently licensed as LeishTec in Brazil which requires further optimization under field conditions [104,105]. Saponin has been also used in formulation with salivary gland extract and *L. braziliensis* proteins (LbSapSal) against CVL exhibiting long-lasting protection [106–108] (Table 1).

6. Particulate adjuvants

6.1. Alum

Alum including a range of aluminium based salts is a classical adjuvant extensively used for around 80 years as it is economical, safe and can be formulated with a range of antigens [114]. Aluminium compounds due to their adsorption property form a short depot at the site of injection thus slowly releasing vaccine antigen to the immune system. The adjuvanticity of alum is linked to NLRPs inflammasome activation and caspase 1 dependent release of IL-1β and IL-18 cytokines [115]. IL-1β promotes CD4 + T-cells differentiation to Th2- and Th17-cells augmenting antibody production (Fig. 1). The inability of alum to induce Th1 biased humoral and cellular response limits its applicability to vaccines against VL. However, alum has been incorporated in combination with other immunomodulators to induce Th1 type response.

6.2. Liposome

Liposomes are spherical vesicles of amphipathic phospholipid bilayers and have been widely used as delivery system for numerous antigens and drugs. These fuse with macrophage membrane inducing both antibody as well as cell-mediated immune responses (CMI). Nkanishi *et al.*, 1999 reported that cationic liposome act as a more potent CMI inducer specifically antigen-specific CTL and DTH responses compared to anionic and neutral liposomes containing the same antigen concentrations. In accordance with this, anionic liposomes encapsulating *L. donovani* promastigote membrane antigens exhibited partial protection reflecting Th2 dominance [116] whereas antigen in cationic liposomes conferred significant protective immunity in BALB/c mice [117]. Another group of workers reported profound adaptive responses in rodents when immunized with *Leishmania* soluble antigen (sLAg) entrapped in escheriosome (non-phosphatidylcholine based liposome) [118]. Furthermore, the potentiating effect of non-coding plasmid DNA against *L. donovani* challenge was significantly enhanced when co-entrapped with soluble antigens in cationic liposomes [119]. Similarly, gp63 of *L. donovani* promastigotes formulated with distearoylphosphatidyl choline (DSPC) cationic liposomes conferred dose-dependent long-term durable protection against progressive VL [120] (Table 2).

6.3. Montanide

Montanide adjuvants are water-in-oil emulsions composed of mineral oil or metabolizable oil mixed with a surfactant and available in different forms [121]. These have been shown to induce high antibody titer and CTL responses in various animal models. In one of the study, histone 1 (H1) and HASPB1 immunized separately, or together as a protein cocktail vaccine with montanide elicited protection in 5/8 in dogs immunized with H1 Montanide, and 4/8 immunized with either HASPB1 combination with Montanide or the cocktail of H1 + HASPB1 with Montanide [122] (Table 2).

6.4. Freund's adjuvants

Freund's adjuvant is a water-in-oil emulsion of mineral oil and is of two types; complete and incomplete. Complete Freund's adjuvant (CFA) includes heat-killed *Mycobacterium* while incomplete Freund's adjuvant (IFA) is without Mycobacteria. Previously, IFA was used in human vaccine formulations like killed poliomyelitis

Table 3
List of adjuvants combinations that have been tested with vaccine candidates against VL.

Adjuvants	Vaccines/Routes of administration	Against Infection	Test Models	Remarks	References
CpG-ODN and Montanide 720	*L. infantum* Cysteine proteinase type I & II (pCB6-cpa and pCB6-cpb)/i.m.	*L. infantum*	BALB/c mice, Dogs	Reduction in hepatic and splenic parasites in mice and bone marrow of dogs remained free of parasites, strong DTH and splenocyte proliferation, ↑ IgG, IgG2a, IFN-γ/IL-5, IFN-γ/IL-10, IFN-γ mRNA in PBMCs	[40,129]
	L. infantum Cysteine proteinase type III (pcDNA-cpc)/s.c.	*L. infantum*	BALB/c mice	Significant parasite reduction in spleen and liver, ↑ NO, IgG2a/IgG1, IFN-γ	[141]
	L. infantum pcDNA-CTE/s.c.	*L. infantum*	BALB/c mice	Couldn't control parasite propagation, ↑ IgG1 and IgG2a, IL-5, IL-5/IFN-γ ratio	[130]
MPL-TDM and Liposome	rGP63/s.c.	*L. donovani*	BALB/c mice	2 and 1.5-log-fold reduced parasite burden in liver and spleen resp., ↑ DTH, IFN-γ, IL-12 p40, IL-4, IgG2a	[131]
	Cysteine proteases cocktail/s.c.	*L. donovani*	Hamster	10^{13}–10^{16} folds parasite reduction, ↑ IFN-γ, IL-2, TNF-α, IL-12, IgG2, NO, DTH, LTT, ↓ IL-4, IL-10	[132]
	Soluble *L. donovani* Ag/s.c.	*L. donovani*	BALB/c mice	89% and 87% protection in liver and spleen resp., ↑DTH, IgG2a/IgG1 ratio, IFN-γ, ↓ IL-4	[133]
Alum and BCG	Alum precipitated ALM and BCG/ i.d.	*L. donovani*	Indian Langur	Complete cure in 87% of animals, + DTH response, 6/7 and 6/8 animals showed + LTT response, ↑ IFN-γ	[134]
			Dogs	69.3% efficacy and decrease in incidence rate from 12% to 3.7% in dogs	[135]
			Human	Safe, no seroconversion, LST conversion rate varies in children from 56% at 6 months to 31% at 2 years	[136]
Alum-BCG-Imiquimod	Alum-ALM-BCG-Imiquimod/i.d.		Dogs	40.04% efficacy rate, 16.3% seroconversion, 30.0% skin test conversion	[137]
Alum + IL-12	Ad5-A2 + rA2/rhIL12/Alum/s.c.	*L. infantum*	Macaque	Lowering of parasite load in liver, pre-challenge production of anti-A2 specific IgG antibodies, hepatic granuloma resolution and reduction of clinical symptoms	[138]
Virosome + GLA-SE	LJL143, KMP11 and LeishF3+/i.m.		BALB/c mice	Safe, ↑ CD4 + and CD8 + cell proliferation, IFN-γ versus IL-10 response, IgG2aα-LJL143 and a mixed IgG1/IgG2a response against LeishF3+	[139]
Nanoparticles-MPL-A	PLGA-soluble *L. infantum* antigens-MPL-A/s.c.	*L. infantum*	BALB/c mice	92% parasite reduction, ↑ IgG2a, Splenocyte proliferation, CD8 + T cell producing IFN-γ, ↓ IL-4 and IL-10	[140]

Footnotes: ↑ – Increase, ↓ – Decrease, resp. – respectively, Ab – antibody, i.d. – intradermal, s.c. – subcutaneous, i.m. – intramuscular, i.p. – intraperitoneal, DTH – Delayed Type Hypersensitivity, LTT – Lymphocyte Transformation Test, NO – Nitric Oxide, LDU – Leishman Donovan Unit, PBMCs – Peripheral Blood Mononuclear Cells.

vaccines and influenza [123]. Emulsions based adjuvants associate with antigen and facilitate its distribution to draining lymph node thereby assisting in interaction with cells of the immune system. It leads to the stimulation of antibody production due to nonspecific immunopotentiation of macrophages [124].

CFA adjuvanticity was evaluated with the recombinant 30 kDa antigen from *L. chagasi* amastigotes provided partial protection too [125]. Other antigens formulated with freund's adjuvants were rLcr1 yielding partial protection and rORFF and rBT1 exhibiting splenocyte proliferation along with the production of antigen-specific antibody [126,127]. Moreover, immunization with acidic ribosomal protein rLdP1 eliciting reduction in parasite burden comparable to infected control but unable to stimulate the release of protective Th1 response significantly [128] (Table 2).

7. Combination of adjuvants for optimization of immunogenicity of candidate vaccines

The increasing availability of new generations of adjuvants in last decade enables development of synergistic adjuvant combinations most likely augmenting their adjuvanticity and eliciting the desired mix of immunological responses. A combination of desired adjuvants requires an understanding of the nature of the immunogen as well as the type of immune response required.

Many combinations of adjuvants have been utilized, (Table 3) for instance, CpG motif adjuvant in conjunction with montanide 720 induces both Th1 biased humoral and cell-mediated immunity thereby providing protection against canine and murine VL [40,129]. However, a mixed type 1 and 2 immune signature was displayed when the combination of these adjuvants was used with C-terminal extension of cysteine proteinase type I [130]. Additionally, liposomal rGp63 and cysteine proteinases in association with MPL-Trehalose Dicorynomycolate (TDM) induce a profound immune response [131,132]. Similarly, liposome encapsulating mixture of leishmanial soluble antigen and MPL-TDM exhibited sustained and long-term protection in comparison to that induced by liposomal SLA [133]. Moreover, a combination of alum and BCG with ALM has been evaluated in different animal models eliciting positive DTH response with better survival and a significant reduction in parasite burden. This preparation was also tried in humans and was completely safe [134–136]. In another study, alum-ALM mixed with BCG and a TLR-7 agonist Imiquimod possessing profound Th1 response was assessed against CVL but its efficacy was low [137]. Another study indicated remarkable clinical and parasitological protection against VL when immunized via heterologous prime-boost regime with recombinant human interleukin 12 and alum [138]. Recently, there is a growing interest in using immunopotentiating influenza virosome as ideal anti-leishmanial vaccine delivery system. Their resemblance with the intact virus and ability of cell-penetration along with immunological properties due to viral proteins altogether provide a novel strategy against VL. One such study reported the safety and immunogenicity of virosome loaded with recombinant proteins LJL143 (from *Lutzomyia longipalpis* saliva), KMP11 and LeishF3 + and adjuvanted with GLA-SE [139]. Recently, biodegradable polymeric nanoparticles have emerged as one of the approaches for confronting VL due to several advantages including enhanced encapsulated antigen stability, safety, biocompatibility, controlled antigen release, and polymer degradation. In a study, poly-(D,L-lactide-co-glycolide) nanospheres surface modified with TNF-α mimicking peptide and entrapping *L. infantum* antigens with MPL-A, conferred remarkable protection against parasite challenge [140]. These results suggest that synergy between different classes of adjuvants could be investigated to augment vaccine efficacy.

Table 4
List of candidate vaccines used with alternative adjuvants in a comparative study against VL.

Antigen	Adjuvants	Infection/ Animal model	Remarks		References
			Parasite reduction	Immunological response	
78 kDa of *L. donovani*/s.c.	rIL-12 Liposome MPLA ALD FCA	*L. donovani*/ BALB/c mice	71–94.8% in spleen 62.9–93.4% in spleen 56.5–92% in spleen 51.3–71% in spleen 49–69% in spleen	↑DTH, IFN-γ, IL-2, IgG2a, ↓ IL-4, IgG1	[142]
Freeze-thawed *L.* antigen/s.c.	Liposome MPLA Saponin Alum	*L. donovani*/ BALB/c mice	78.7–90% in liver 75.9–88.8% in liver 74.2–84.1% in liver 66.8–81.08% in liver	↑DTH, IFN-γ, IL-12, IgG2a, ↓ IL-10, IL-4, IgG1	[144]
Killed *L. donovani*/s.c.	Liposome MPLA Saponin Alum	*L. donovani*/ BALB/c mice	86–93.7% in liver 83.4–92.8% in liver 79.9–88.8% in liver 73.78–82.9% in liver	↑ DTH, IFN-γ, IL-12, IgG2a, ↓ IL-10, IL-4, IgG1	[68]
Autoclaved *L. donovani*/s.c.	Liposome MPLA Saponin Alum	*L. donovani*/ BALB/c mice	79.06–91.6% in liver 77.5–90.8% in liver 75.6–85.6% in liver 69.8–79.2% in liver	↑ DTH, IFN-γ, IL-12, IgG2a, ↓ IL-10, IL-4, IgG1	[143]
Chimerical Protein Q/i.d.	Freund's adjuvant/Aluminium hydroxide/HSP 70/BCG/CpG-ODN/ pCDNA3/pUC18	*L. infantum*/ BALB/c mice	Q/CpG-ODN conferred maximum 99% parasite reduction in liver and spleen	↑ IgG2a/IgG1 ratio, IFN-γ, ↓ IL-4	[145]
FML/s.c.	Quil A	*L. donovani*/ Swiss Albino mice	93% in liver	↑ anti-FML IgG1, IgG2a, IgG2b, + DTH	[88]
	QS21		79.2% in liver	↑ anti-FML IgG1, IgG2a, IgG2b, + DTH, IFN-γ	
	Riedel De Haën Saponin pure		73% in liver	↑ anti-FML IgG1, IgG2a, IgG2b, + DTH, IFN-γ	
	BCG		52% in liver	↑ anti-FML IgG and IgM responses (minor and non-specific protection), IFN-γ	
	IL-12		No parasite reduction	↑ anti-FML IgG1, IgG2a, IgG2b, + DTH	
L. donovani promastigote antigen/i.p.	BCG/MPL-TDM/Cationic Liposome	*L. donovani*/ BALB/c mice	Cationic liposome conferred highest parasite reduction in liver and spleen	↑ DTH, IgG2a, IFN-γ, ↓ IL-4	[146]
L. donovani pSP/ s.c.	IL-12	*L. donovani*/ BALB/c mice	91% and 89% parasite reduction in spleen and liver resp.	↑ Splenocytes proliferation, DTH, TNF-α, IFN-γ, ↓ IL-4, IL-10	[62]
	Freund's Complete Adjuvant		82% and 79% parasite reduction in spleen and liver resp.		
L. donovani sonicated antigen/i.d.	Alum-BCG/Montanide ISA/MPL-A	*L. donovani*/ Vervet monkey	Alum-BCG and Montanide ISA conferred significant parasite reduction in spleen	↑ IgG2, ↓ IL-4, IL-10	[147]

Footnotes: ↑ – Increase, ↓ – Decrease, resp. – respectively, Ab – antibody, i.d. – intradermal, s.c. – subcutaneous, i.m. – intramuscular, i.p. – intraperitoneal, DTH – Delayed Type Hypersensitivity, LTT – Lymphocyte Transformation Test, NO – Nitric Oxide, LDU – Leishman Donovan Unit, PBMCs – Peripheral Blood Mononuclear Cells.

8. Comparative effectiveness of different adjuvant systems

The use of right adjuvant is of paramount importance in order to develop an effective vaccine. Various studies have investigated the comparative analysis of different adjuvants in formulation with a vaccine candidate (Table 4). Immunogenicity of 78 kDa antigen formulated with different adjuvants was evaluated, out of which the highest level of protection was observed with 78 kDa + rIL-12 [67,142]. Thakur et al. in 2015 demonstrated maximum protection with the use of liposome encapsulated killed *L. donovani*, freeze-thawed *L.* antigen, and autoclaved *L. donovani* when compared with other adjuvants [68,143,144]. CpG-ODN was evaluated prophylactically against VL by comparison against alternative adjuvants with Protein Q in a murine model and it was observed that CpG-ODN revealed the highest level of protection as compared to other formulations [145]. Furthermore, Moreover, a strong protective effect was observed against murine VL when immunized with FML in conjunction with QuilA saponin [88]. In a comparative study, the highest level of protection was exhibited by liposomal *Leishmania* antigen against murine VL [146]. Similarly, *L. donovani* secretory serine proteases (pSP) conferred maximum protection when delivered with IL-12 adjuvant following *L. donovani* challenge [62]. A study indicated that alum-BCG (AIBCG), as well as montanide ISA 720 with *L. donovani* sonicate antigen induces low Th2 cytokines and high IgG2 antibodies in the vervet monkey model [147].

9. Conclusion

Despite enormous efforts in the field of advancement towards vaccine adjuvants and enhanced understanding of VL pathogenesis, there is no vaccine licensed for immunoprophylaxis or immunotherapy against human VL. Recent development in adjuvant biology and vaccine development anticipates the search for an adjuvant capable of eliciting a stronger immune response against anthroponotic VL. With this concept, several human clinical trials were carried out where BCG, MPL-SE, and GLA-SE adjuvants were used in vaccine formulations, out of which few have reached to phase I clinical trial and emerged as potential adjuvants. However, their translation against natural disease condition in

Table 5
List of human vaccine adjuvants that have been clinically tested.

Adjuvants	Formulations	Clinical trials	Mode of action	References
MF59	Squalene-based oil-in-water emulsion	Approved for H5N1 pandemic influenza vaccine and H1N1 influenza vaccine	Induces high antibody titre with balanced IgG1:IgG2 ratio	[148,149]
AS03	Squalene-based oil-in-water emulsion containing α-tocopherol	Used in licensed influenza vaccine	Induces antibody response and cell-mediated immunity	[150]
AS04	MPL adsorbed to aluminium salts	Approved for HPV and HBV vaccines	Induces humoral and Th1 type of immune responses	[151]
Virosome	A reconstituted membrane of an enveloped virus	Approved for influenza virus as well as HAV vaccines	Induces antibody and CTL responses	[152]
Alum	Aluminium based salts	Included in licensed human vaccines like HAV, HBV, HPV, diphtheria, tetanus, Haemophilus Influenzae Type b (Hib) and meningococcal.	Induces humoral and Th2 type of immune responses	[114,153]
Poly Inosinic: Cytidilic	TLR-3 agonist	Phase I trial of H5N1 influenza virus vaccine	Induces strong humoral, Th1 type and CTL responses	[154]
Flagellin	TLR-5 agonist	Phase I trial of Influenza virus vaccine	Induces humoral, Th1 and Th2 type of immunity	[154]
CpG	TLR-9 agonist	Phase III trial of HBV vaccine	Induces strong humoral, Th1 type and CTL responses	[154,8]
AS01	Combination of liposome, MPL, QS-21	Phase III trial of Malaria and Cancer vaccines	Induces strong humoral, Th1 type and CTL responses	[154,8]
AS02	Oil in water emulsion containing MPL and QS-21	Phase I-III trials of Malaria, Tuberculosis, HPV, HBV, HIV	Induces potent humoral and Th1 responses	[154,8]
ISCOM and ISCOMMATRIX	Lipid-based adjuvant containing antigen, saponin, cholesterol and phospholipid	Phase I-III trials of Influenza, HPV, HCV	Induces humoral, Th1 and Th2 type of immunity and CTL response	[154]

Footnotes: HPV – Human Papilloma Virus, HAV – Hepatitis A Virus, HBV – Hepatitis B Virus, HCV – Hepatitis C Virus, ISCOM – Immune Stimulating Complex, CTL – Cytotoxic T-lymphocyte.

humans is yet to be seen. While, in case of zoonotic VL, LeishTec (rA2 protein with saponin) and Leishmune (FML protein in combination with Quil-A saponin) are the two currently available licensed commercial vaccines in Brazil, giving rise to a ray of hope towards the approval and licensing of vaccines for human VL also. Although a plethora of adjuvants has been proposed in these years for use with potential *Leishmania* vaccines, they could not be exploited commercially perhaps due to difficulty in designing of potent, safe and economically feasible adjuvants. It may be worth exploiting a handful of clinically validated vaccine adjuvant formulations viz., MF59, AS03, AS04 and virosomes that have been licensed for human use against influenza virus, hepatitis virus, human papilloma virus (HPV) and cancer [8,148–155] (Table 5). The success of clinical trials conducted with these novel adjuvants formulations demonstrates that synergy between multiple adjuvants targeting different arms of the immune system is critically important for rational vaccine design. Therefore, these potent adjuvants formulations should be able to confer long-term parasite-specific cellular immune response and will hopefully lead to approval for vaccines against human VL.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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References

[1] Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, et al. Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? Nat Rev Microbiol 2007;5:873–82. https://doi.org/10.1038/nrmicro1748.

[2] McQuarrie S, Kasper K, Moffatt DC, Marko D, Keynan Y. Relapse of visceral leishmaniasis in an HIV-infected patient successfully treated with a combination of miltefosine and amphotericin B. Can J Infect Dis Med Microbiol 2015;26:325–9. https://doi.org/10.1155/2015/176545.

[3] Ramesh V, Singh R, Avishek K, Verma A, Deep DK, Verma S, et al. decline in clinical efficacy of oral miltefosine in treatment of post kala-azar dermal leishmaniasis (PKDL) in India. PLoS Negl Trop Dis 2015;9:1–12. https://doi.org/10.1371/journal.pntd.0004093.

[4] Bhandari V, Kulshrestha A, Deep DK, Stark O, Prajapati VK, Ramesh V, et al. Drug susceptibility in Leishmania isolates following Miltefosine treatment in cases of Visceral Leishmaniasis and post Kala-Azar dermal Leishmaniasis. PLoS Negl Trop Dis 2012;6:1–6. https://doi.org/10.1371/journal.pntd.0001657.

[5] Raman VS, Duthie MS, Fox CB, Matlashewski G, Reed SG. Adjuvants for Leishmania vaccines: from models to clinical application. Front Immunol 2012;3:1–15. https://doi.org/10.3389/fimmu.2012.00144.

[6] Maisonneuve C, Bertholet S, Philpott DJ, De Gregorio E. Unleashing the potential of NOD- and Toll-like agonists as vaccine adjuvants. Proc Natl Acad Sci 2014;111:12294–9. https://doi.org/10.1073/pnas.1400478111.

[7] Gupta RK, Relyveld EH, Lindblad EB, Bizzini B, Ben-Efraim S, Gupta CK. Adjuvants – a balance between toxicity and adjuvanticity. Vaccine 1993;11:293–306. https://doi.org/10.1016/0264-410X(93)90190-9.

[8] Reed SG, Bertholet S, Coler RN, Friede M. New horizons in adjuvants for vaccine development. Trends Immunol 2009;30:23–32. https://doi.org/10.1016/j.it.2008.09.006.

[9] Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. Nat Med 2013;19:1597–608. https://doi.org/10.1038/nm.3409.

[10] Ansari NA, Saluja S, Salotra P. Elevated levels of interferon-γ, interleukin-10, and interleukin-6 during active disease in Indian kala azar. Clin Immunol 2006;119:339–45. https://doi.org/10.1016/j.clim.2006.01.017.

[11] Nylen S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. Trends Immunol 2007;28:378–84. https://doi.org/10.1016/j.it.2007.07.004.

[12] Gautam S, Kumar R, Maurya R, Nylén S, Ansari N, Rai M, et al. IL-10 neutralization promotes parasite clearance in splenic aspirate cells from patients with visceral leishmaniasis. J Infect Dis 2011;204:1134–7. https://doi.org/10.1093/infdis/jir461.

[13] Pitta MGR, Romano A, Cabantous S, Henri S, Hammad A, Kouriba B, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Invest 2009;119:2379–87. https://doi.org/10.1172/JCI38813.

[14] Nascimento MSL, Carregaro V, Lima-Júnior DS, Costa DL, Ryffel B, Duthie MS, et al. Interleukin 17A acts synergistically with interferon γ to promote protection against leishmania infantum infection. J Infect Dis 2015;211:1015–26. https://doi.org/10.1093/infdis/jiu531.

[15] Kumar R, Nylén S. Immunobiology of visceral leishmaniasis. Front Immunol 2012;3:1–10. https://doi.org/10.3389/fimmu.2012.00251.
[16] Ansari NA, Kumar R, Gautam S, Nylen S, Singh OP, Sundar S, et al. IL-27 and IL-21 are associated with T Cell IL-10 Responses in Human Visceral Leishmaniasis. J Immunol 2011;186:3977–85. https://doi.org/10.4049/jimmunol.1003588.
[17] Spolski R, Zhu W, Leonard WJ, Kim H-P, Levy DE. IL-21 Mediates suppressive effects via its induction of IL-10. J Immunol 2009;182:2859–67. https://doi.org/10.4049/jimmunol.0802978.
[18] Rai AK, Thakur CP, Singh A, Seth T, Srivastava SK, Singh P, et al. Regulatory T cells suppress T cell activation at the pathologic site of human visceral leishmaniasis. PLoS One 2012;7(2):e31551. https://doi.org/10.1371/journal.pone.0031551.
[19] Kaushal H, Bras-Goncaalves R, Negi NS, Lemesre JL, Papierok G, Salotra P. Role of CD8+T cells in protection against Leishmania donovani infection in healed Visceral Leishmaniasis individuals. BMC Infect Dis 2014;14:8–14. https://doi.org/10.1186/s12879-014-0653-6.
[20] Gautam S, Kumar R, Singh N, Singh AK, Rai M, Sacks D, et al. CD8 T cell exhaustion in human visceral leishmaniasis. J Infect Dis 2014;209:290–9. https://doi.org/10.1093/infdis/jit401.
[21] Silva-Barrios S, Smans M, Duerr CU, Qureshi ST, Fritz JH, Descoteaux A, et al. Innate immune B cell activation by leishmania donovani exacerbates disease and mediates hypergammaglobulinemia. Cell Rep 2016;15:2427–37. https://doi.org/10.1016/j.celrep.2016.05.028.
[22] Ghosh AK, Dasgupta S, Ghose AC. Immunoglobulin G subclass-specific antileishmanial antibody responses in Indian kala-azar and post-kala-azar dermal leishmaniasis. Clin Diagn Lab Immunol 1995;2:291–6.
[23] Ryan JR, Smithyman AM, Rajasekariah GH, Hochberg L, Stiteler JM, Martin SK. Enzyme-linked immunosorbent assay based on soluble promastigote antigen detects immunoglobulin M (IgM) and IgG antibodies in sera from cases of visceral and cutaneous leishmaniasis. J Clin Microbiol 2002;40:1037–43. https://doi.org/10.1128/JCM.40.3.1037-1043.2002.
[24] Kedzierski L. Leishmaniasis vaccine: where are we today? J Glob Infect Dis 2010;2:177–85. https://doi.org/10.4103/0974-777X.62881.
[25] Souza AE, Bates PA, Coombs GH, Mottram JC. Null mutants for the lmcpa cysteine proteinase gene in Leishmania mexicana. Mol Biochem Parasitol 1994;63:213–20. https://doi.org/10.1016/0166-6851(94)90057-4.
[26] El Fadili A, Kündig C, Roy G, Ouellette M. Inactivation of the leishmania tarentolae pterin transporter (BT1) and reductase (PTR1) genes leads to viable parasites with changes in folate metabolism and hypersensitivity to the antifolate methotrexate. J Biol Chem 2004;279:18575–82. https://doi.org/10.1074/jbc.M400652200.
[27] Fiuza JA, Dey R, Davenport D, Abdeladhim M, Meneses C, Oliveira F, et al. Intradermal immunization of leishmania donovani centrin knock-out parasites in combination with salivary protein LJM19 from sand fly vector induces a durable protective immune response in hamsters. PLoS Negl Trop Dis 2016;10:1–17. https://doi.org/10.1371/journal.pntd.0004322.
[28] Carrillo E, Crusat M, Nieto J, Chicharro C, Thomas MdelC, Martínez E, et al. Immunogenicity of HSP-70, KMP-11 and PFR-2 leishmanial antigens in the experimental model of canine visceral leishmaniasis. Vaccine 2008;26:1902–11. https://doi.org/10.1016/j.vaccine.2008.01.042.
[29] Ghosh A, Zhang WW, Matlashewski G. Immunization with A2 protein results in a mixed Th1/Th2 and a humoral response which protects mice against Leishmania donovani infections. Vaccine 2001;20:59–66. https://doi.org/10.1016/S0264-410X(01)00322-X.
[30] Goto Y, Bogatzki LY, Bertholet S, Coler RN, Reed SG. Protective immunization against visceral leishmaniasis using Leishmania sterol 24-c-methyltransferase formulated in adjuvant. Vaccine 2007;25:7450–8. https://doi.org/10.1016/j.vaccine.2007.08.001.
[31] Stäger S, Smith DF, Kaye PM. Immunization with a recombinant stage-regulated surface protein from Leishmania donovani induces protection against visceral leishmaniasis. J Immunol 2000;165:7064–71. https://doi.org/10.4049/jimmunol.165.12.7064.
[32] Tewary P, Jain M, Sahani MH, Saxena S, Madhubala R. A heterologous prime boost vaccination regimen using ORFF DNA and recombinant ORFF protein confers protective immunity against experimental visceral leishmaniasis. J Infect Dis 2005;191:2130–7. https://doi.org/10.1086/430348.
[33] Coler RN, Goto Y, Bogatzki L, Raman V, Reed SG. Leish-111f, a recombinant polyprotein vaccine that protects against visceral leishmaniasis by elicitation of CD4+T cells. Infect Immun 2007;75:4648–54. https://doi.org/10.1128/IAI.00394-07.
[34] Chakravarty J, Kumar S, Trivedi S, Rai VK, Singh A, Ashman JA, et al. A clinical trial to evaluate the safety and immunogenicity of the LEISH-F1+MPL-SE vaccine for use in the prevention of visceral leishmaniasis. Vaccine 2011;29:3531–7. https://doi.org/10.1016/j.vaccine.2011.02.096.
[35] Coler RN, Duthie MS, Hofmeyer KA, Guderian J, Jayashankar L, Vergara J, et al. From mouse to man: safety, immunogenicity and efficacy of a candidate leishmaniasis vaccine LEISH-F3+GLA-SE. Clin Transl Immunol 2015;4:. https://doi.org/10.1038/cti.2015.6e35.
[36] Duthie MS, Pereira L, Favila M, Hofmeyer KA, Reed SJ, Metangmo S, et al. A defined subunit vaccine that protects against vector-borne visceral leishmaniasis. npj Vaccines 2017;2:23. https://doi.org/10.1038/s41541-017-0025-5.
[37] Marques-Da-Silva EA, Coelho EAF, Gomes DCO, Vilela MC, Masioli CZ, Tavares CAP, 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. Parasitol Res 2005;98:67–74. https://doi.org/10.1007/s00436-005-0008-8.
[38] Gamboa-León R, Paraguai de Souza E, Borja-Cabrera GP, Santos FN, Myashiro LM, Pinheiro RO, et al. Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase-DNA vaccine of Leishmania donovani. Vaccine 2006;24:4863–73. https://doi.org/10.1016/j.vaccine.2006.03.005.
[39] Sharma A, Madhubala R. Ubiquitin conjugation of open reading frame F DNA vaccine leads to enhanced cell-mediated immune response and induces protection against both antimony-susceptible and -resistant strains of leishmania donovani. J Immunol 2009;183:7719–31. https://doi.org/10.4049/jimmunol.0900132.
[40] Rafati S, Nakhaee A, Taheri T, Taslimi Y, Darabi H, Eravani D, 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 2005;23:3716–25. https://doi.org/10.1016/j.vaccine.2005.02.009.
[41] Guha R, Das S, Ghosh J, Naskar K, Mandala A, Sundar S, et al. Heterologous priming-boosting with DNA and vaccinia virus expressing kinetoplastid membrane protein-11 induces potent cellular immune response and confers protection against infection with antimony resistant and sensitive strains of Leishmania (Leishmania. Vaccine 2013;31:1905–15. https://doi.org/10.1016/j.vaccine.2013.02.025.
[42] Badiee A, Heravi Shargh V, Khamesipour A, Jaafari MR. Micro/nanoparticle adjuvants for antileishmanial vaccines: present and future trends. Vaccine 2013;31:735–49. https://doi.org/10.1016/j.vaccine.2012.11.068.
[43] Satti INY, Osman H, Daifalla NS, Younis SA, Khalil EAG, Zijlstra EE, et al. Immunogenicity and safety of autoclaved Leishmania major plus BCG vaccine in healthy Sudanese volunteers. Vaccine 2001;19:2100–6. https://doi.org/10.1016/S0264-410X(00)00401-1.
[44] Srivastava JK, Misra A, Sharma P, Srivastava B, Naik S, Dube A. Prophylactic potential of autoclaved Leishmania donovani with BCG against experimental visceral leishmaniasis. Parasitology 2003;127:107–14. https://doi.org/10.1017/S0031182003003457.
[45] Ferreira JHL, Gentil LG, Dias SS, Fedeli CEC, Katz S, Barbiéri CL. Immunization with the cysteine proteinase Ldccys1 gene from Leishmania (Leishmania) chagasi and the recombinant Ldccys1 protein elicits protective immune responses in a murine model of visceral leishmaniasis. Vaccine 2008;26:677–85. https://doi.org/10.1016/j.vaccine.2007.11.044.
[46] Tripathi P, Gupta SK, Sinha S, Sundar S, Dube A, Naik S. Prophylactic efficacy of high-molecular-weight antigenic fractions of a recent clinical isolate of Leishmania donovani against visceral leishmaniasis. Scand J Immunol 2008;68:492–501. https://doi.org/10.1111/j.1365-3083.2008.02171.x.
[47] Kumari S, Samant M, Misra P, Khare P, Sisodia B, Shasany AK, et al. Th1-stimulatory polyproteins of soluble Leishmania donovani promastigotes ranging from 89.9 to 97.1 kDa offers long-lasting protection against experimental visceral leishmaniasis. Vaccine 2008;26:5700–11. https://doi.org/10.1016/j.vaccine.2008.08.021.
[48] Gupta R, Kushawaha PK, Tripathi CDP, Sundar S, Dube A. A novel recombinant Leishmania donovani p45, a partial coding region of methionine aminopeptidase, generates protective immunity by inducing a Th1 stimulatory response against experimental visceral leishmaniasis. Int J Parasitol 2012;42:429–35. https://doi.org/10.1016/j.ijpara.2012.02.013.
[49] Khare P, Jaiswal AK, Tripathi CDP, Joshi S, Sundar S, Dube A. Efficacy of Leishmania donovani trypanothione reductase, identified as a potent Th1 stimulatory protein, for its immunogenicity and prophylactic potential against experimental visceral leishmaniasis. Parasitol Res 2014;113(3):851–62. https://doi.org/10.1007/s00436-013-3716-5.
[50] Gupta R, Kumar V, Kushawaha PK, Tripathi CP, Joshi S, Sahasrabuddhe AA, et al. Characterization of glycolytic enzymes – rAldolase and rEnolase of Leishmania donovani, identified as Th1 stimulatory proteins, for their immunogenicity and immunoprophylactic efficacies against experimental visceral leishmaniasis. PLoS One 2014;9:e86073. https://doi.org/10.1371/journal.pone.0086073.
[51] Baharia RK, Tandon R, Sharma T, Suthar MK, Das S, Siddiqi MI, et al. Recombinant NAD-dependent SIR-2 Protein of leishmania donovani: immunobiochemical characterization as a potential vaccine against visceral leishmaniasis. PLoS Negl Trop Dis 2015;9:1–26. https://doi.org/10.1371/journal.pntd.0003557.
[52] Jaiswal AK, Khare P, Joshi S, Rawat K, Yadav N, Sundar S, et al. Immunological consequences of stress-related proteins-cytosolic tryparedoxin peroxidase and chaperonin TCP20-identified in splenic amastigotes of Leishmania donovani as Th1 stimulatory, in experimental visceral leishmaniasis. Parasitology 2015;142:728–44. https://doi.org/10.1017/S003118201400184X.
[53] Khare P, Jaiswal AK, Tripathi CDP, Sundar S, Dube A. Immunoprotective responses of T helper type 1 stimulatory protein-S-adenosyl-L-homocysteine hydrolase against experimental visceral leishmaniasis. Clin Exp Immunol 2016;185:165–79. https://doi.org/10.1111/cei.12780.
[54] Tandon R, Chandra S, Baharia RK, Misra P, Das S, Rawat K, et al. Molecular, biochemical characterization and assessment of immunogenic potential of cofactor-independent phosphoglycerate mutase against Leishmania donovani: a step towards exploring novel vaccine candidate. Parasitology 2018;145:508–26. https://doi.org/10.1017/S0031182017001160.
[55] Ananias RZ, Rodrigues EG, Braga EG, Squaiella CC, Mussalem JS, Longhini ALF, et al. Modulatory effect of killed Propionibacterium acnes and its purified soluble polysaccharide on peritoneal exudate cells from C57Bl/6 mice: Major

NKT cell recruitment and increased cytotoxicity. Scand J Immunol 2007;65:538–48. https://doi.org/10.1111/j.1365-3083.2007.01939.x.
[56] Ferreira JHL, Silva LdosS, Longo-Maugéri IM, Katz S, Barbiéri CL. Use of a recombinant cysteine proteinase from leishmania (Leishmania) infantum chagasi for the immunotherapy of canine visceral leishmaniasis. PLoS Negl Trop Dis 2014;8:1–8. https://doi.org/10.1371/journal.pntd.0002729.
[57] Ogawa C, Liu Y-J, Kobayashi KS. Muramyl dipeptide and its derivatives: peptide adjuvant in immunological disorders and cancer therapy. Curr Bioact Compd 2011;7:180–97. https://doi.org/10.2174/157340711796817913.
[58] Lemesre JL, Holzmuller P, Cavaleyra M, Gonçalves RB, Hottin G, Papierok G. Protection against experimental visceral leishmaniasis infection in dogs immunized with purified excreted secreted antigens of Leishmania infantum promastigotes. Vaccine 2005;23:2825–40. https://doi.org/10.1016/j.vaccine.2004.11.061.
[59] Bourdoiseau G, Hugnet C, Gonçalves RB, Vézilier F, Petit-Didier E, Papierok G, et al. Effective humoral and cellular immunoprotective responses in Li ESAp-MDP vaccinated protected dogs. Vet Immunol Immunopathol 2009;128:71–8. https://doi.org/10.1016/j.vetimm.2008.10.309.
[60] Lemesre JL, Holzmuller P, Gonçalves RB, Bourdoiseau G, Hugnet C, Cavaleyra M, et al. Long-lasting protection against canine visceral leishmaniasis using the LiESAp-MDP vaccine in endemic areas of France: Double-blind randomised efficacy field trial. Vaccine 2007;25:4223–34. https://doi.org/10.1016/j.vaccine.2007.02.083.
[61] Bliss J1, Van Cleave V, Murray K, Wiencis A, Ketchum M, Maylor R, Haire T, Resmini C, Abbas AKWS. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. J Immunol 1996;156:887–94.
[62] Choudhury R, Das P, De T, Chakraborti T. 115kDa serine protease confers sustained protection to visceral leishmaniasis caused by Leishmania donovani via IFN-γ induced down-regulation of TNF-α mediated MMP-9 activity. Immunobiology 2013;218:114–26. https://doi.org/10.1016/j.imbio.2012.02.008.
[63] Poot J, Spreeuwenberg K, Sanderson SJ, Schijns VECJ, Mottram JC, Coombs GH, 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 2006;24:2460–8. https://doi.org/10.1016/j.vaccine.2005.12.039.
[64] Badaró R, Nascimento C, Carvalho JS, Badaró F, Russo D, Ho JL, et al. Granulocyte-macrophage colony-stimulating factor in combination with pentavalent antimony for the treatment of visceral leishmaniasis. Eur J Clin Microbiol Infect Dis 1994;13:23–8. https://doi.org/10.1007/BF01973598.
[65] Badaro R, Lobo I, Nakatani M, Muiños A, Netto EM, Coler RN, et al. Successful use of a defined antigen/GM-CSF adjuvant vaccine to treat mucosal Leishmaniasis refractory to antimony: A case report. Braz J Infect Dis 2001;5:223–32. https://doi.org/10.1590/S1413-86702001000400008.
[66] Sundar S, Singh VP, Sharma S, Makharia MK, Murray HW. Response to interferon-gamma plus pentavalent antimony in Indian visceral leishmaniasis. J Infect Dis 1997;176:1117–9. https://doi.org/10.1086/520665.
[67] Nagill R, Kaur T, Joshi J, Kaur S. Immunogenicity and efficacy of recombinant 78 kDa antigen of Leishmania donovani formulated in various adjuvants against murine visceral leishmaniasis. Asian Pac J Trop Med 2015;8:513–9. https://doi.org/10.1016/j.apjtm.2015.06.008.
[68] Thakur A, Kaur H, Kaur S. Evaluation of the immunogenicity and protective efficacy of Killed Leishmania donovani antigen along with different adjuvants against experimental visceral leishmaniasis. Med Microbiol Immunol 2015;204:539–50. https://doi.org/10.1007/s00430-014-0367-9.
[69] Bhardwaj S, Vasishta RK, Arora SK. Vaccination with a novel recombinant Leishmania antigen plus MPL provides partial protection against L. donovani challenge in experimental model of visceral leishmaniasis. Exp Parasitol 2009;121:29–37. https://doi.org/10.1016/j.exppara.2008.09.019.
[70] Fujiwara RT, Vale AM, França Da Silva JC, Da Costa RT, Quetz JDS, Martins Filho OA, et al. Immunogenicity in dogs of three recombinant antigens (TSA, LelF and LmSTI1) potential vaccine candidates for canine visceral leishmaniasis. Vet Res 2005. https://doi.org/10.1051/vetres: 2005033.
[71] Gradoni L, Foglia Manzillo V, Pagano A, Piantedosi D, De Luna R, Gramiccia M, et al. Failure of a multi-subunit recombinant leishmanial vaccine (MML) to protect dogs from Leishmania infantum infection and to prevent disease progression in infected animals. Vaccine 2005;23:5245–51. https://doi.org/10.1016/j.vaccine.2005.07.001.
[72] Trigo J, Abbehusen M, Netto EM, Nakatani M, Pedral-Sampaio G, de Jesus RS, et al. Treatment of canine visceral leishmaniasis by the vaccine Leish-111f +MPL-SE. Vaccine 2010;28:3333–40. https://doi.org/10.1016/j.vaccine.2010.02.089.
[73] Goto Y, Bhatia A, Raman VS, Liang H, Mohamath R, Picone AF, et al. KSAC, the first defined polyprotein vaccine candidate for visceral leishmaniasis. Clin Vaccine Immunol 2011;18:1118–24. https://doi.org/10.1128/CVI.05024-11.
[74] Roatt BM, Aguiar-Soares RDde O, Reis LES, Cardoso JMdeO, Mathias FAS, de Brito RCF, et al. A vaccine therapy for canine visceral leishmaniasis promoted significant improvement of clinical and immune status with reduction in parasite burden. Front Immunol 2017;8. https://doi.org/10.3389/fimmu.2017.00217.
[75] Miret J, Nascimento E, Sampaio W, França JC, Fujiwara RT, Vale A, et al. Evaluation of an immunochemotherapeutic protocol constituted of N-methyl meglumine antimoniate (Glucantime®) and the recombinant Leish-110f® + MPL-SE® vaccine to treat canine visceral leishmaniasis. Vaccine 2008;26:1585–94. https://doi.org/10.1016/j.vaccine.2008.01.026.
[76] Joshi J, Kaur S. To investigate the therapeutic potential of immunochemotherapy with cisplatin + 78 kDa + MPL-A against Leishmania donovani in BALB/c mice. Parasite Immunol 2014;36:3–12. https://doi.org/10.1111/pim.12071.
[77] Joshi J, Kaur S. Studies on the protective efficacy of second-generation vaccine along with standard antileishmanial drug in Leishmania donovani infected BALB/c mice. Parasitology 2014;141:554–62. https://doi.org/10.1017/S0031182013001959.
[78] Coler RN, Bertholet S, Moutaftsi M, Guderian JA, Windish HP, Baldwin SL, et al. Development and characterization of synthetic glucopyranosyl lipid adjuvant system as a vaccine adjuvant. PLoS One 2011;6:1–12. https://doi.org/10.1371/journal.pone.0016333.
[79] Duthie MS, Favila M, Hofmeyer KA, Tutterrow YL, Reed SJ, Laurance JD, et al. Strategic evaluation of vaccine candidate antigens for the prevention of Visceral Leishmaniasis. Vaccine 2016;34:2779–86. https://doi.org/10.1016/j.vaccine.2016.04.067.
[80] Tewary P, Sukumaran B, Saxena S, Madhubala R. Immunostimulatory oligodeoxynucleotides are potent enhancers of protective immunity in mice immunized with recombinant ORFF leishmanial antigen. Vaccine 2004;22:3053–60. https://doi.org/10.1016/j.vaccine.2004.02.007.
[81] Tewary P, Mehta J, Sukumaran B, Madhubala R. Vaccination with Leishmania soluble antigen and immunostimulatory oligodeoxynucleotides induces specific immunity and protection against Leishmania donovani infection. FEMS Immunol Med Microbiol 2004;42:241–8. https://doi.org/10.1016/j.femsim.2004.05.008.
[82] Mazumder S, Maji M, Das A, Ali N. Potency, efficacy and durability of DNA/DNA, DNA/protein and protein/protein based vaccination using gp63 against Leishmania donovani in BALB/c mice. PLoS One 2011:6. https://doi.org/10.1371/journal.pone.0014644.
[83] Ramirez L, Corvo L, Duarte MC, Chávez-Fumagalli MA, Valadares DG, Santos DM, et al. Cross-protective effect of a combined L5 plus L3 Leishmania major ribosomal protein based vaccine combined with a Th1 adjuvant in murine cutaneous and visceral leishmaniasis. Parasites Vectors 2014;7:1–11. https://doi.org/10.1186/1756-3305-7-3.
[84] Datta N, Mukherjee S, Das L, Das PK. Targeting of immunostimulatory DNA cures experimental visceral leishmaniasis through nitric oxide up-regulation and T cell activation. Eur J Immunol 2003;33:1508–18. https://doi.org/10.1002/eji.200323671.
[85] Shivahare R, Vishwakarma P, Parmar N, Yadav PK, Haq W, Srivastava M, et al. Combination of liposomal CpG oligodeoxynucleotide 2006 and miltefosine induces strong cell-mediated immunity during experimental visceral leishmaniasis. PLoS One 2014;9:1–12. https://doi.org/10.1371/journal.pone.0094596.
[86] Sun HX, Xie Y, Ye YP. Advances in saponin-based adjuvants. Vaccine 2009;27:1787–96. https://doi.org/10.1016/j.vaccine.2009.01.091.
[87] Borja-Cabrera GP, Mendes AC, Paraguai De Souza E, Okada LYH, Trivellato FADA, Kawasaki JKA, et al. Effective immunotherapy against canine visceral leishmaniasis with the FML-vaccine. Vaccine 2004;22:2234–43. https://doi.org/10.1016/j.vaccine.2003.11.039.
[88] Santos WR, De Lima VMF, De Souza EP, Bernardo RR, Palatnik M, De Sousa CBP. Saponins, IL12 and BCG adjuvant in the FML-vaccine formulation against murine visceral leishmaniasis. Vaccine 2002;21:30–43. https://doi.org/10.1016/S0264-410X(02)00444-9.
[89] Oliveira-Freitas E, Casas CP, Borja-Cabrera GP, Santos FN, Nico D, Souza LOP, et al. Acylated and deacylated saponins of Quillaja saponaria mixture as adjuvants for the FML-vaccine against visceral leishmaniasis. Vaccine 2006;24:3909–20. https://doi.org/10.1016/j.vaccine.2006.02.034.
[90] Palatnik De Sousa CB, Santos WR, Casas CP, Paraguai De Souza E, Tinoco LW, Da Silva BP, et al. Protective vaccination against murine visceral leishmaniasis using aldehyde-containing Quillaja saponaria sapogenins. Vaccine 2004;22:2470–9. https://doi.org/10.1016/j.vaccine.2004.01.072.
[91] Santos WR, Aguiar IA, de Souza E Paraguai, de Lima VM, Palatnik M, Palatnik-de-Sousa CB. Immunotherapy against murine experimental visceral leishmaniasis with the FML-vaccine. Vaccine 2003 [pii] S0264410X03005279.
[92] Borja-Cabrera GP, Correia Pontes NN, Da Silva VO, Paraguai De Souza E, Santos WR, Gomes EM, et al. lasting protection against canine kala-azar using the FML-QuilA saponin vaccine in an endemic area of Brazil (São Gonçalo do Amarante, RN). Vaccine 2002;20:3277–84. https://doi.org/10.1016/S0264-410X(02)00294-3.
[93] Santos FN, Borja-Cabrera GP, Miyashiro LM, Grechi J, Reis AB, Moreira MAB, et al. Immunotherapy against experimental canine visceral leishmaniasis with the saponin enriched-Leishmune®vaccine. Vaccine 2007;25:6176–90. https://doi.org/10.1016/j.vaccine.2007.06.005.
[94] Borja-Cabrera GP, Santos FN, Bauer FS, Parra LE, Menz I, Morgado AA, et al. Immunogenicity assay of the Leishmune vaccine against canine visceral leishmaniasis in Brazil. Vaccine 2008;26:4991–7. https://doi.org/10.1016/j.vaccine.2008.07.029.
[95] Martins VT, Chávez-Fumagalli MA, Costa LE, Martins AMCC, Lage PS, Lage DP, et al. Antigenicity and protective efficacy of a leishmania amastigote-specific protein, member of the super-oxygenase family, against visceral leishmaniasis. PLoS Negl Trop Dis 2013:7. https://doi.org/10.1371/journal.pntd.0002148.
[96] Martins VT, Chávez-Fumagalli MA, Lage DP, Duarte MC, Garde E, Costa LE, et al. Antigenicity, immunogenicity and protective efficacy of three proteins expressed in the promastigote and amastigote stages of leishmania infantum

against visceral leishmaniasis. PLoS One 2015;10:1–25. https://doi.org/10.1371/journal.pone.0137683.
[97] Martins VT, Lage DP, Duarte MC, Costa LE, Garde E, Rodrigues MR, et al. A new Leishmania-specific hypothetical protein, LiHyT, used as a vaccine antigen against visceral leishmaniasis. Acta Trop 2016;154:73–81. https://doi.org/10.1016/j.actatropica.2015.11.006.
[98] Martins VT, Duarte MC, Chávez-Fumagalli MA, Menezes-Souza D, Coelho CSP, De Magalhães-Soares DF, et al. A Leishmania-specific hypothetical protein expressed in both promastigote and amastigote stages of Leishmania infantum employed for the serodiagnosis of, and as a vaccine candidate against, visceral leishmaniasis. Parasites Vectors 2015;8:1–12. https://doi.org/10.1186/s13071-015-0964-5.
[99] Dias DS, Martins VT, Ribeiro PAF, Ramos FF, Lage DP, Tavares GSV, et al. Antigenicity, immunogenicity and protective efficacy of a conserved Leishmania hypothetical protein against visceral leishmaniasis. Parasitology 2018;145:740–51. https://doi.org/10.1017/S0031182017001731.
[100] Lage DP, Martins VT, Duarte MC, Garde E, Chávez-Fumagalli MA, Menezes-Souza D, et al. Prophylactic properties of a Leishmania-specific hypothetical protein in a murine model of visceral leishmaniasis. Parasite Immunol 2015;37:646–56. https://doi.org/10.1111/pim.12287.
[101] Duarte MC, Lage DP, Martins VT, Costa LE, Lage LMR, Carvalho AMRS, et al. A vaccine combining two Leishmania braziliensis proteins offers heterologous protection against Leishmania infantum infection. Mol Immunol 2016;76:70–9. https://doi.org/10.1016/j.molimm.2016.06.014.
[102] Santos TTO, Martins VT, Lage DP, Costa LE, Salles BCS, Carvalho AMRS, et al. Probing the efficacy of a heterologous Leishmania/L. Viannia braziliensis recombinant enolase as a candidate vaccine to restrict the development of L. infantum in BALB/c mice. Acta Trop 2017;171:8–16. https://doi.org/10.1016/j.actatropica.2017.03.008.
[103] Dias DS, Ribeiro PAF, Martins VT, Lage DP, Portela ÁSB, Costa LE, et al. Recombinant small glutamine-rich tetratricopeptide repeat-containing protein of Leishmania infantum: potential vaccine and diagnostic application against visceral leishmaniasis. Mol Immunol 2017;91:272–81. https://doi.org/10.1016/j.molimm.2017.09.017.
[104] Fernandes AP, Costa MMS, Coelho EAF, Michalick MSM, de Freitas E, Melo MN, et al. Protective immunity against challenge with Leishmania (Leishmania) chagasi in beagle dogs vaccinated with recombinant A2 protein. Vaccine 2008;26:5888–95. https://doi.org/10.1016/j.vaccine.2008.05.095.
[105] Grimaldi G, Teva A, Dos-Santos CB, Santos FN, Pinto IDS, Fux B, et al. Field trial of efficacy of the Leish-tec® vaccine against canine leishmaniasis caused by Leishmania infantum in an endemic area with high transmission rates. PLoS One 2017;12:1–18. https://doi.org/10.1371/journal.pone.0185438.
[106] Resende LA, Aguiar-Soares RDDO, Gama-Ker H, Roatt BM, De Mendonça LZ, Alves MLR, et al. Impact of LbSapSal vaccine in canine immunological and parasitological features before and after leishmania chagasi-challenge. PLoS One 2016;11:1–21. https://doi.org/10.1371/journal.pone.0161169.
[107] Aguiar-Soares RDDO, Roatt BM, Ker HG, Moreira NDD, Mathias FAS, Cardoso JMDO, et al. LBSapSal-vaccinated dogs exhibit increased circulating T-lymphocyte subsets (CD4+and CD8+) as well as a reduction of parasitism after challenge with Leishmania infantum plus salivary gland of Lutzomyia longipalpis. Parasites Vectors 2014;7:1–10. https://doi.org/10.1186/1756-3305-7-61.
[108] Giunchetti RC, Corrêa-Oliveira R, Martins-Filho OA, Teixeira-Carvalho A, Roatt BM, de Aguiar-Soares RD, et al. A killed Leishmania vaccine with sand fly saliva extract and saponin adjuvant displays immunogenicity in dogs. Vaccine 2008;26:623–38. https://doi.org/10.1016/j.vaccine.2007.11.057.
[109] Santos WR, de Souza E Paraguai, Palatnik M, Palatnik de Sousa CB. Vaccination of Swiss Albino mice against experimental visceral leishmaniasis with the FML antigen of Leishmania donovani. Vaccine 1999;17:2554–61. https://doi.org/10.1016/S0264-410X(99)00058-4.
[110] Paraguai de Souza E, Bernardo RR, Palatnik M, Palatnik de Sousa CB. Vaccination of Balb/c mice against experimental visceral leishmaniasis with the GP36 glycoprotein antigen of Leishmania donovani. Vaccine 2001;19:3104–15. https://doi.org/10.1016/S0264-410X(01)00031-7.
[111] Chávez-Fumagalli MA, Costa MAF, Oliveira DM, Ramírez L, Costa LE, Duarte MC, et al. Vaccination with the Leishmania infantum ribosomal proteins induces protection in BALB/c mice against Leishmania chagasi and Leishmania amazonensis challenge. Microbes Infect 2010;12:967–77. https://doi.org/10.1016/j.micinf.2010.06.008.
[112] Nico D, Claser C, Borja-Cabrera GP, Travassos LR, Palatnik M, da Soares I, et al. Adaptive immunity against leishmania nucleoside hydrolase maps its C-terminal domain as the target of the CD4+ T cell-driven protective response. PLoS Negl Trop Dis 2010. https://doi.org/10.1371/journal.pntd.0000866.
[113] Nico D, Morrot A, Palatnik-de-Sousa CB. Vaccination with nucleoside hydrolase (NH36) of L. (L.) donovani or its C-terminal portion (F3) in formulation with saponin prevents the increase of the proportions of spleen dendritic cells in murine experimental visceral leishmaniasis. Proc Vaccinol 2014;8:58–61. https://doi.org/10.1016/j.provac.2014.07.010.
[114] Kool M, Fierens K, Lambrecht BN. Alum adjuvant: some of the tricks of the oldest adjuvant 2018:927–34. https://doi.org/10.1099/jmm.0.038943-0.
[115] Burns K, Martinon F, Tschopp J. New insights into the mechanism of IL-1β maturation. Curr Opin Immunol 2003;15:26–30. https://doi.org/10.1016/S0952-7915(02)00017-1.
[116] Afrin F, Anam K, Ali N. Induction of partial protection against Leishmania donovani by promastigote antigens in negatively charged liposomes. J Parasitol 2000;86:730–5. https://doi.org/10.1645/0022-3395(2000)086[0730:IOPPAL]2.0.CO;2.
[117] Afrin F, Rajesh R, Anam K, Gopinath M, Pal S, Ali N. Characterization of Leishmania donovani antigens encapsulated in liposomes that induce protective immunity in BALB/c mice. Infect Immun 2002;70:6697–706. https://doi.org/10.1128/IAI.70.12.6697-6706.2002.
[118] Sharma SK, Dube A, Nadeem A, Khan S, Saleem I, Garg R, et al. Non PC liposome entrapped promastigote antigens elicit parasite specific CD8+and CD4+T-cell immune response and protect hamsters against visceral leishmaniasis. Vaccine 2006;24:1800–10. https://doi.org/10.1016/j.vaccine.2005.10.025.
[119] Mazumder S, Ravindran R, Banerjee A, Ali N. Non-coding pDNA bearing immunostimulatory sequences co-entrapped with leishmanial antigens in cationic liposomes elicits almost complete protection against experimental visceral leishmaniasis in BALB/c mice. Vaccine 2007;25:8771–81. https://doi.org/10.1016/j.vaccine.2007.10.028.
[120] Bhowmick S, Ravindran R, Ali N. Gp63 in stable cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with Leishmania donovani. Infect Immun 2008;76:1003–15. https://doi.org/10.1128/IAI.00611-07.
[121] Aucouturier J, Dupuis L, Deville S, Ascarateil S, Ganne V. Montanide ISA 720 and 51: a new generation of water in oil emulsions as adjuvants for human vaccines. Expert Rev Vaccines 2002;1:111–8. https://doi.org/10.1586/14760584.1.1.111.
[122] Moreno J, Nieto J, Masina S, Cañavate C, Cruz I, Chicharro C, et al. Immunization with H1, HASPB1 and MML Leishmania proteins in a vaccine trial against experimental canine leishmaniasis. Vaccine 2007;25:5290–300. https://doi.org/10.1016/j.vaccine.2007.05.010.
[123] Salk J, Salk D. Control of influenza and poliomyelitis with killed virus vaccines. Science(80–) 1977. https://doi.org/10.1126/science.320661.
[124] Mutiso JM, Macharia JC, Gicheru MM. A review of adjuvants for vaccine candidates. J Biomed Res 2010;24:16–25.
[125] Pinto AR, Beyrodt CGP, Lopes RAM, Barbiéri CL. Identification of a 30 kDa antigen from Leishmania (L.) chagasi amastigotes implicated in protective cellular reponses in a murine model. Int J Parasitol 2000;30:599–607. https://doi.org/10.1016/S0020-7519(00)00037-0.
[126] Ali KM, Donelson JE, Wilson ME, Young BM, Andersen KP, Weinstock JV, et al. A recombinant leishmania chagasi antigen that stimulates cellular immune responses in infected mice 1995;63:2062–9.
[127] Dole VS, Raj VS, Ghosh A, Madhubala R, Myler PJ, Stuart KD. Immunization with recombinant LD1 antigens protects against experimental leishmaniasis. Vaccine 2000;19:423–30. https://doi.org/10.1016/S0264-410X(00)00207-3.
[128] Masih S, Arora SK, Vasishta RK. Efficacy of Leishmania donovani ribosomal P1 gene as DNA vaccine in experimental visceral leishmaniasis. Exp Parasitol 2011;129:55–64. https://doi.org/10.1016/j.exppara.2011.05.014.
[129] Rafati S, Zahedifard F, Nazgouee F. Prime-boost vaccination using cysteine proteinases type I and II of Leishmania infantum confers protective immunity in murine visceral leishmaniasis. Vaccine 2006;24:2169–75. https://doi.org/10.1016/j.vaccine.2005.11.011.
[130] Rafati S, Zahedifard F, Azari MK, Taslimi Y, Taheri T. Leishmania infantum: prime boost vaccination with C-terminal extension of cysteine proteinase type I displays both type 1 and 2 immune signatures in BALB/c mice. Exp Parasitol 2008;118:393–401. https://doi.org/10.1016/j.exppara.2007.10.004.
[131] Mazumder S, Maji M, Ali N. Potentiating effects of MPL on DSPC bearing cationic liposomes promote recombinant GP63 vaccine efficacy: high immunogenicity and protection. PLoS Negl Trop Dis 2011;5:e1429. https://doi.org/10.1371/journal.pntd.0001429.
[132] Das A, Ali N. Combining cationic liposomal delivery with MPL-TDM for cysteine protease cocktail vaccination against leishmania donovani: evidence for antigen synergy and protection. PLoS Negl Trop Dis 2014;8:e3091. https://doi.org/10.1371/journal.pntd.0004185.
[133] Ravindran R, Maji M, Ali N. Vaccination with liposomal leishmanial antigens adjuvanted with monophosphoryl lipid-trehalose dicorynomycolate (MPL-TDM) confers long-term protection against visceral leishmaniasis through a human administrable route. Mol Pharm 2012;9:59–70. https://doi.org/10.1021/mp2002494.
[134] Misra A, Dube A, Srivastava B, Sharma P, Srivastava JK, Katiyar JC, et al. Successful vaccination against Leishmania donovani infection in Indian langur using alum-precipitated autoclaved Leishmania major with BCG. Vaccine 2001;19:3485–92. https://doi.org/10.1016/S0264-410X(01)00058-5.
[135] Mohebali M, Khamesipour A, Mobedi I, Zarei Z, Hashemi-Fesharki R. Double-blind randomized efficacy field trial of alum precipitated autoclaved Leishmania major vaccine mixed with BCG against canine visceral leishmaniasis in Meshkin-Shahr district, I.R. Iran. Vaccine 2004;22:4097–100. https://doi.org/10.1016/j.vaccine.2004.03.058.
[136] Khalil EAG, Musa AM, Modabber F, El-Hassan AM. Safety and immunogenicity of a candidate vaccine for visceral leishmaniasis (Alum-precipitated autoclaved Leishmania major + BCG) in children: an extended phase II study. Ann Trop Paediatr 2006;26:357–61. https://doi.org/10.1179/146532806X152890.
[137] Barati M, Mohebali M, Alimohammadian MH, Khmesipour A, Keshavarz H, Akhoundi B, et al. Double-blind randomized efficacy field trial of alum precipitated autoclaved Leishmania major (Alum-ALM) vaccine mixed with BCG plus imiquimod vs. placebo control group. Iran J Parasitol 2015;10:351–9.

[138] Jr Grimaldi G, Teva A, Porrozzi R, Pinto MA, Marchevsky RS, Rocha MGL, et al. Clinical and parasitological protection in a leishmania infantum-macaque model vaccinated with adenovirus and the recombinant A2 antigen. PLoS Negl Trop Dis 2014;8:e2853. https://doi.org/10.1371/journal.pntd.0002853.
[139] Cecílio P, Pérez-Cabezas B, Fernández L, Moreno J, Carrillo E, Requena JM, et al. Pre-clinical antigenicity studies of an innovative multivalent vaccine for human visceral leishmaniasis. PLoS Negl Trop Dis 2017;11:1–26. https://doi.org/10.1371/journal.pntd.0005951.
[140] Margaroni M, Agallou M, Athanasiou E, Kammona O, Kiparissides C, Gaitanaki C, et al. Vaccination with poly(D, L-lactide-co-glycolide) nanoparticles loaded with soluble Leishmania antigens and modified with a TNFalpha-mimicking peptide or monophosphoryl lipid A confers protection against experimental visceral leishmaniasis. Int J Nanomed 2017;12:6169–84. https://doi.org/10.2147/IJN.S141069.
[141] Khoshgoo N, Zahedifard F, Azizi H, Taslimi Y, Alonso MJ, Rafati S. Cysteine proteinase type III is protective against Leishmania infantum infection in BALB/c mice and highly antigenic in visceral leishmaniasis individuals. Vaccine 2008;26:5822–9. https://doi.org/10.1016/j.vaccine.2008.08.065.
[142] Nagill R, Kaur S. Enhanced efficacy and immunogenicity of 78kDa antigen formulated in various adjuvants against murine visceral leishmaniasis. Vaccine 2010;28:4002–12. https://doi.org/10.1016/j.vaccine.2010.01.015.
[143] Thakur A, Kaur H, Kaur S. Evaluation of the immunoprophylactic potential of a killed vaccine candidate in combination with different adjuvants against murine visceral leishmaniasis. Parasitol Int 2015;64:70–8. https://doi.org/10.1016/j.parint.2014.10.003.
[144] Thakur A, Kaur H, Kaur S. Studies on the protective efficacy of freeze thawed promastigote antigen of Leishmania donovani along with various adjuvants against visceral leishmaniasis infection in mice. Immunobiology 2015;220:1031–8. https://doi.org/10.1016/j.imbio.2015.05.014.
[145] Parody N, Soto M, Requena JM, Álonso C. 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 Immunol 2004;26:283–93. https://doi.org/10.1111/j.0141-9838.2004.00711.x.
[146] Ravindran R, Bhowmick S, Das A, Ali N. Comparison of BCG, MPL and cationic liposome adjuvant systems in leishmanial antigen vaccine formulations against murine visceral leishmaniasis. BMC Microbiol 2010;10:181. https://doi.org/10.1186/1471-2180-10-181.
[147] Mutiso JM, Macharia JC, Gicheru MM. Immunization with leishmania vaccine-alum-BCG and montanide ISA 720 adjuvants induces low-grade type 2 cytokines and high levels of IgG2 subclass antibodies in the vervet monkey (Chlorocebus aethiops) model. Scand J Immunol 2012;76:471–7. https://doi.org/10.1111/j.1365-3083.2012.02764.x.
[148] O'Hagan DT, Ott GS, Van Nest G, Rappuoli R, Del Giudice G. The history of MF59 ® adjuvant: a phoenix that arose from the ashes. Expert Rev Vaccines 2013;12:13–30. https://doi.org/10.1586/erv.12.140.
[149] Durando P, Icardi G, Ansaldi F. MF59-adjuvanted vaccine: a safe and useful tool to enhance and broaden protection against seasonal influenza viruses in subjects at risk. Expert Opin Biol Ther 2010;10:639–51. https://doi.org/10.1517/14712591003724662.
[150] Atmar RL, Keitel WA. Adjuvants for pandemic influenza vaccines. Curr Top Microbiol Immunol 2009;333:323–44. https://doi.org/10.1007/978-3-540-92165-3_16.
[151] Garcon N, Wettendorff M, Van Mechelen M. Role of AS04 in human papillomavirus vaccine: mode of action and clinical profile. Expert Opin Biol Ther 2011;11:667–77. https://doi.org/10.1517/14712598.2011.573624.
[152] Schwendener RA. Liposomes as vaccine delivery systems: a review of the recent advances. Ther Adv Vaccines 2014;2:159–82. https://doi.org/10.1177/2051013614541440.
[153] Oleszycka E, Lavelle EC. Immunomodulatory properties of the vaccine adjuvant alum. Curr Opin Immunol 2014;28:1–5. https://doi.org/10.1016/j.coi.2013.12.007. doi:10.1016/j.coi.2013.12.007.
[154] Lee S, Nguyen MT. Recent advances of vaccine adjuvants for infectious diseases. Immune Netw 2015;15:51. https://doi.org/10.4110/in.2015.15.2.51.