Review Article

Immunotherapy and Immunochemotherapy for Leishmaniasis: An Overview

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ABSTRACT

Instead of relying on drugs to reduce the parasitic burden of leishmaniasis and waiting for the effecter immune response to develop in time to control the parasities, immunotherapy in conjugation with chemotherapy can rapidly induce the effector immune response. With a safe and potent drug plus an affordable therapeutic vaccine, which remains to be developed, a single visit by patient with leishmaniasis might be sufficient to induce a quick and lasting recovery. Human vaccination against leishmaniasis using live leishmania was used in middle east and Russia (1941-1980). First generation vaccines composed by killing parasites induce low efficacies (54%). Second generation vaccines using live genetically modified parasites or bacteria or viruses containing leishmania genes, the use of adjuvants increased vaccine efficacies of the purified antigens to 82%. Recombinant second generation vaccines and third generation DNA vaccines showed average values of parasitic load reduction of 68% and 59% in laboratory animal models respectively, but their success in field trials had not yet been reported. To date only three vaccines have been licenced for use one in Uzbekistan and two in Brazil. Drug toxicity and the emergence of resistance could also be dramatically reduced compared with present long term monotherapy. Immunotherapy could be an effective addition to chemotherapy for leishmaniasis.

Keywords : leishmaniasis, Immunotherapy, Chemotherapy, Immunochemotherapy.

INTRODUCTION

Leishmaniasis is a disease caused by several digenetic protozoan species of genus *leishmania*, which affect humans and different domestic animals. The disease may arise in different forms and the most important are: visceral leishmaniasis, cutaneous leishmaniasis and mucocutaneous leishmaniasis. Visceral leishmaniasis is most dangerous as it affects liver and spleen. It is also known as kala-azar and is characterised by high fever, substantial weight loss, swelling of spleen and liver. If left untreated, the disease can have fatality rate as high as 100% within two years[1]. The carrier of parasite is female phlebotomine commonly known as sandfly[2]. Approximately 350 million people in eight countries are estimated to be threatened by the disease. The world health organisation estimated that there are 12 million cases of all forms of leishmaniasis worldwide with more than 500000 new cases of visceral disease occurring each year[1]. Bihar state in India alone accounts for 50% of the world burden of visceral leishmaniasis[3]. Visceral leishmaniasis is caused by *leishmania donovani* (Africa, India, Asia), *l. chagasi*(America) and *l. infantum* (Mediterranean basin). Cutaneous leishmaniasis is produced by *l. Mexicana* and *l. braziliensis* complexes in America and *l. major, l. tropica* and *l. aethiopica* in old world[4,5].

In the vertebrate host, leishmania parasite survives and multiplies intercellularly in mononuclear phagocytes as nonmotile amastigotes, about 2 to 4 µm in diameter. However recent evidence indicates that cells other than mononuclear phagocytes, for example fibroblasts may also harbour parasite [6]. Transmission to the vertebrate host is from phlebotomine sandfly in which parasite developand replicate as 20 µm flagellated promastigotes. Inside the fly the parasite undergo a developmental program starting with the amastigote in the blood meal, continuing through several stages of promastigote maturation and culminating with the infectious metacyclic form. The environmental cues which trigger this program are not well understood, but temperature and pH appears to play a role.

Leishmaniasis is considered a zoonosis and humans are generally accidental hosts. An important exception to the zoonotic character of leishmaniasis is that the reservoir for cutaneous disease caused by *l. tropica* in the middle east and visceral disease in India is probably made up of other infected humans. The animal reservoir shows geographic variation and include rodents, dogs and other mammals.

The first vaccine against leishmaniasis was developed by prof. Alder at the Hebrew University of Jerusalem, Israel. WHO had observed that mothers of Lebanon exposed their children arms to the bite of sand flies because they intuitively knew that the development of a self healing single first lesion would protect them from several disease in future[7]. Therefore the ancient practice was to inoculate uninfected individuals with infectious Material from lesions, in region of body where the scar would be hidden[7]. After a method for axenic culture of the parasite was established "leishmanization" because usual in Israel and Russia and further evoluted to the use of first generation vaccine composed of whole killed parasite or crude extracts. Leishmanization process was discontinued due to uncontrolled long lasting skin lesions, the spread of HIV and the use of immunosuppressive drugs, ethical reasons, parasite persistence and difficulties, in the quality inoculums control. Its use at present is limited to one vaccine registered in Uzbekistan and to live challenge in vaccine efficacy trials in humans in Iran[8]. First generation leishmania vaccines is composed of killed parasite[9] having gradually replaced leishmanization. Second generation vaccines thus far have been based on the following : live, genetically modified *leishmania* species designed to cause abortive infection in man, recombinant bacteria or viruses carrying *leishmania* antigen genes, defined synthetic or recombinant subunits[10,11] and native fractions purified from parasites[12,13]. The use of third generation vaccines[9] that includes genes coding for a protective antigen, cloned into a vector containing eukaryotic promoter is the more recent approach[14,15,16].

Until recently, only two vaccines (one live and one killed) were licensed for use in humans and for prophylaxis dogs[17]. Also the number of available drugs for treating the disease is limited and most of the parasite develop drug resistancy. Over the last two decades, immunotherapy, either alone or combined with chemotherapy (immunochemotherapy), has been developed as an additional approach in the treatment of leishmaniasis. Immunotherapy has been used to accelerate the specific immune response in immunologically responsive patients and to establish an effective reaction in those who are non-responsive[18].

Immunology of leishmaniasis

The response of the immune system to *leishmania* infection are highly complex. They may accelerate cure or exacerbate the disease, depending on the particular circumstances. This is partially due to the effects of genetic variation in the mammalian host, partially due to genetic variation in the parasites between species and strains, and partially due to chance factors such as the location, inoculums size and number of infective bites received[19,20]. The innate immune response to *leishmania* is mediated by natural killing (NK) cells, cytokines and mononuclear and polymorphnuclear phagocytes. The production of IL-12 early in the course of infection by dendritic cells, leads to the early activation of NK cells and the production of IFN-γ[21]. The early NK-cell activation is also induced by chemokines (IP-10, MCP-1 and lymphotactin). Activated NK-cell derived IFN-Y plays a more prominent role in host defence by activating macrophages to kill the intracellular parasite through the generation of reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI). Activated polymorphonuclear leucocytes kill parasite primarily through oxidative mechanism. Recently it was shown that IFN-α and TEN-β participate in the early induction of NO and control of parasite replication early in infection[22]. In an endemic area the prevalence of DTH (delayed type hypersensitivity) skin test positively increases and the incidence of clinical disease with age, indicating the acquisition of immunity in the population over time. Retrospective epidemiological studies indicate that most individual with prior infection (subclinical or healed) are immune to a subsequent clinical infection[23]. There is extensive evidence from experimental models that cellular immune mechanisms mediated acquired resistance to *leishmania* infection and human studies have generally confirmed this[6]. Antileishmanial antibodies, which are produced at a low levelin CL and at a very high level in VL, play no role in protection. A high antibody level is a marker of progressive disease in VL[23].

Although there are some differences among the different *leishmania* species, the general mechanism of cellular immunity can be summarised. Following infection in the skin, langerhans cells phagocytose and transport *leishmania* to the regional lymph nodes, where they induce a T-cell response. Acquired immunity in murine cutaneous leishmaniasis caused by *l. major* is mediated by parasite-induced production of IFN-γ by CD4 T cells (th 1 subset), and can develop in absence of CD8 T cells[24]. Both CD4 and CD8 T cells are required for an effective defence against murine visceral *l. Donovani* infections, but the precise role of CD8 Tcells is unclear[23].

However, in murine visceral *l. Infanatum* infection, CD8cells played multiple roles comprising both cytotoxic activity against cells expressing *leishmania* antigens and secretion of cytokines and chemokines[25]. The generation of th 1 response is IL-12 dependent[21] and the generation of IL-12 is critically dependent on signalin from CD40 to its ligand, CD40-L (CD153). Depletion of IL-12, or disruption of IL-12 gene, IL-18 gene or STAT 4 gene (critical to IL-12 signaling) subverts th 1 cell development and renders resistant mice susceptible. Administration of IL-12 is protective as long as it is given early in the course of infection. Tumor necrosis factor-α (TNF-α) contributes to protective immunity by synergizing with IFN-γ to activate macrophages[23]. The generation of RNI (reactive nitrogen intermediate) by activating macrophages is the primary mechanism of parasite killing in murine model. The importance of NO as an antileishmanial effector mechanism is underscored by the following observations: (1) the killing of parasite by IFN-γ activated macrophages in –vitro is dependent on expression of nitric oxide synthase (iNOS) and the generation of NO (2) leishmania-resistant mouse strains demonstrate high level of iNOS expression and NO generation and are rendered susceptible when iNOS is inhibited and (3) mice carrying a null deletion of the NOS2 gene are highly susceptible to leishmanial infection. Although IFN-γ mediated generation of NO plays a prominent role in control of infection in mouse models, its role in the killing of *leishmania* by human macrophages is unclear[26]. Peripheral blood mononuclear cells (PBMCs) isolated from patients with localised or subclinical leishmaniasis demonstrate a Th1 response to *leishmania* antigens. Patients with ML exhibit vigorous T cell responses, it is postulated that this hyperresponsive state contributes to the prominent tissue distruction of ML[23]. The progression of murine *L.major* infection has been correlated with the expansion of Th2 cells and the production of IL-4, IL-5 and IL-10[24]. IL-4 production within the first day of infection was shown to down-regulate IL-12 receptor B-chain expression and derive the response to a Th2 phenotype. The recognition of a single epitope in the LACK (leishmania-activated C-kinase) antigen by CD4 T cells is responsible for the early IL-4 response in BALB/C mice infected with *L.major*. Neutralization of IL-4 or deletion of the IL-4 gene was shown in a number of studies to convert genetically susceptible mice to a resistant phenotype, however, susceptibility to some *L.major* strains is not strictly mediated by IL-4, IL-13 signaling probably plays a role in the IL-4 independent susceptibility. The production of TGF-β by infected macrophages is also associated with inhibition of IFN-γ production, suppression of macrophage activation and progressive disease. In vitro neutralization of TGF-β was found to promote the healing of *L.major* and *L.amazonensis* infections[23,27]. Patients with DCL resemble the progressive infection caused by *L.major* in BALB/C mice. Such patients demonstrate minimal or absent leishmania-specific lymphoproliferative responses, and the Th2 cytikine mRNAs were prominently expressed in DCL lesions. During active VL in humans there is marked depression of *leishmania*-specific lymphoproliferative responses and IFN-γ responses, as well as an absence of DTH response to parasite antigens this energy appears to be mediated, at least in part, by a suppressive effect of IL-10 and low levels of IL-12. Treatment of active disease restores an antigen-specific Th1 response, and drug treatment of patients with refractory disease can be enhanced by coadministration of IFN-γ[23]. Macrophage mediated suppression leads to the increase in parasite burden and antigen energy, and is linked to either defective antigen presentation or inhibition of expression of class I and class II major histocompatibility complex molecules[28]. Immunosuppression in mice is related to enhance TGF-β expression[29], IL-10 and the possible participation of CD4+, CD25+ regulatory cells. There are two receptors for B-7 molecule the CD28 for Tcell activation (Th 1) and the CTLA-4 for the termination of T cell activation. Blockade of CTLA-4 leads to resistance to infection, suggesting that the expression of CTLA-4 plays an important role in maintaining unresponsiveness in CD4+ T cells during chronical VL in the mouse model[30]. The expression of CTLA-4 results in the secretion of TGF-β, which promotes the growing of the parasite inside the macrophage. Human kala-azar is characterized by high titers of *leishmania*-specific antibodies appearing soon after infection and before the development of cellular immunological abnormalities. The role of these antibodies in disease resolution or protection is largely unknown. The Th 1 cytokine IFN-γ probably upregulates IgG1 and IgG3 in humans, while the Th 2 cytokines IL-4 and IL-5 stimulate the production high level of IgM, IgE and IgG isotypes such as IgG4. Analysis of the *leishmania* specific Ig isotype in

and IgG subclasses in VL patients sera revealed elevated levels of IgG, IgM, IgE and IgG subclasses during disease[31,32,33,34]. Drug resistance was associated with a reduction in IgG2 and IgG3. A marked elevation of IgG1, however was observed in all these patients[31,35]. A successful cure corresponded with a decline, most significantly, in the levels of IgE, IgG4 and IgG1[31,32,33].

History of leishmania vaccines

Historically, cutaneous leishmaniasis has been the focus of vaccination attempts, probably because it has been known since antiquity that individuals who had healed their skin lesions were protected from further infections. Bedouin or some Kurdistani tribal societies traditionally expose their babies bottom to sandfly bites in order to protect them from facial lesions. Another ancient technique practized in middle east has been the use of a thorn to transfer infectious material from lesions to uninfected individuals[36]. With the establishment by Nicolle in 1908 of culture conditions able to support the growth of promastigotes, live organisms started to be used for vaccination. Large scale vaccination trials using live promastigotes were carried out in the soviet union and Israel[37,38] with a high percentage of successful lesion development. The success of this strategy depended critically on the viability and infectivity of the infected organisms. Organisms which had lost virulence were shown to induced delayed type hypersensitivity but did not protect from subsequent natural infection[39]. The use of vaccines had many problems, including the development of large uncontrolled skin lesions, exacerbation of psoriasis and other skin disease, and even immunosuppression as determined by low response to the diphtheria, pertissis and tetanus triple vaccine[9,40]. Consequently the use of live virulent organism for vaccination was discontinued and in the 1990s the focus shifted to killed organisms[36]. The concept of a *leishmania* killed vaccine was neglected for many years, possibly because of conflicting results obtained in the 1940s. Vaccination with killed organism failed to protect person in the middle east[41]. Whereas a brazilian trial showed excellent protection[42,43]. The tide turned when studiesperformed in the 1980s showed that injection of irradiated parasite induced excellent protection in mice provided that they were injected intravenously or intraperitoneally but not subcutaneously. These experiments paved the way for a reassessment of the use of killed vaccines and led to the successful development and field trials of several formulations of killed vaccines[44,45,46].

First generation vaccines

This includes the trials of using the whole living virulent organism, attenuated or killed promastigotes, which were used with or without adjuvants[9]. The use of living vaccines or leishmanization is based on the convalescent immunity which is acquired following induction of a lesion at a selected site with small dose of a cutaneous strains. Leishmanization has been practised for hundreds of years, especially in the middle east and parts of the soviet union. It is a currently used practice in Iran, where a massive program was begun in 1982 after outbreaks of CL among military personnel. As shown in the soviet union and Iran, leishmanization can protect 90% or more of the population from multiple cutaneous lesions or lesions on the face[47]. Unfortunately, leishmanization using virulent strains can be associated with several problems including the development of large slowly healing lesions, lesion contamination and exacerbation of other dermatologic conditions[48]. For these reasons the practice has been restricted in the soviet union and discontinued in Israel. The human efficacy trials involving the so called “Killed vaccines”, composed of crude total parasite antigens, started in Brazil in the 1940s[42]. Most trials were developed against CL[49,50] while only one was directed against VL.

In brazil and Ecuador, extensive vaccination trials have demonstrated that a cocktail of five killed *leishmania* stocks or a single strain of *L. amazonensis* induces significant protection from natural infection[9]. These studies also indicated that delayed type hypersensitivity skin test conversion can be used as a surrogate marker for protective immunity. Moreover the immunized individuals developed long lasting specific T-cell responses of the Th 1 type, which may indicate a potential to protect from infection[51]. In Iran Ali & Afrin(1997) prepared a vaccine against *leishmania donovani*, they used leishmanial antigen to immunize BALB/C mice, by intraperitoneal injection of 20 µg of the antigen in phosphate buffer saline (PBS) and they got a significant level of protection against the disease upto 4 months. An interesting observation is that this vaccine produce a predominantly CD8+ T cell response, like that seen in leishmaniasis patients after their successful treatment[51]. In venzuela, convict and colleagues, some of the early pioneers of killed vaccines used a combination of killed *L. Mexicana* or *L. braziliensis* promastigotes and

M. bovis BCG both prophylactically and therapeutically against South American Leishamaniasis. When used in the therapeutic mode, vaccination appeared to induce a high cure rate even in patients with sever cases. Cure was accompanied by the development of Th 1 type immune response in the recipients with the production of IFN-γ and absence of IL-4. In India, vaccination of Langur monkeys against *L. donovani* by using autoclaved *L. major* plus BCG was tried by Dube et al.,1998. Triple doses each of 1mg killed *L. major* (ALM) plus 1 mg BCG intradermally were tested and found effective for approximately 8 months. This study indicated that a combination of ALM plus BCG may be a good candidate vaccine against human Kala-azar.

Since the average vaccine efficacy value (VE) was relatively low (54.38%), the number of individuals tested was large. Autoclaving of the killed parasite vaccine was introduced[52,53] as the best form of sterilization and preservation of vaccines in countries that have rudimentary biotechnology industry and where a cold chain for distribution is not feasible. The work of De-Luca et al. in 1999 proved however, that as expected for a protozoa parasite, autoclaving destroys most of the proteins of the parasite and the vaccine loose immunogenicity. The most striking aspect of first generation human vaccines is that a leishmanin skin test (LST) is used for candidate selection and for confirmation of immunogenicity[54]. Whenever the LST is performed, VE is obtained among the individuals whose skin tested positive[53,54], whereas no efficacy is detected in assays that did not include an LST[49]. The first generation vaccines were also used against canine visceral leishmaniasis (CVL) inducing protection in Iran but failing to do so in Brazil. Results of phase I trials of first generation human vaccines are also available[52] and they showed that immunogenicity is obtained after a single vaccine dose. Furthermore these vaccines have been used for immunotherapy against human CL with success in Brazil and Venezuela. Although differing in the use of BCG adjuvant and in the number of doses, the authors of both studies agree that the vaccine should not be used as monotherapy but instead, combined with the usual chemotherapy, in order to reduce toxic and very painful antimonial treatment[55]. Based on these results, a first generation vaccine has been registered as adjunct to antimony therapy in Brazil[55].

Second generation vaccines

Live vaccines

This category includes vaccines made of genetically modified,‘‘knock-out’’ *Leishmania* spp., which lack essential genes, such as dyhydrofolate-reductase thymidilate synthasecystein-proteinase[56] or biopterin transporter[57]. These parasites undergo a short life cycle, enough to generate a specific immune response causing abortive infection and no disease in man. However, the useof live challenge for humans is considered ethically unacceptable[58], and an artificial challenge cannot providethe valuable information obtained by exposure to natural infection, which is modulated by components of sand flysaliva[59]. On the other hand, the large number of participantsneeded for a field trial, due to the low expected vaccine efficacy , can be reduced only by the use of vaccineswith higher efficacies, such as second-generation ones formulated with potent adjuvants. In summary, the use of attenuated organism is very attractive because they are the closest mimic to the natural course of infection and may therefore lead to similar immune responses. Moreover, because of the small load of antigen delivery by the transient infection, the immune responses may be skewed even more towards a Th 1 protective response than in natural infections[60]. Such immunization will also deliver many more parasite antigen than the limited number possible with subunit or recombinant antigens. Summarizing a large amount of experimental evidence. Rivier et al., conclude that injection of attenuated organisms achieved better protection than any method involving recombinant gp63 as test antigen delivered with a variety of adjuvants and delivery systems.

Synthetic vaccines

nother approach to second-generation vaccines is to use live recombinant bacteria or virus expressing *Leishmania* parasite antigen; the bacteria and virus serve as expression carrier and adjuvant system. These vaccines have limited practical application. Examples of bacteria vaccines are: *L. major* GP63 surface protease, a major *Leishmania* antigen, cloned in *Salmonella thypymurium* mutant[61] or in BCG; the LCR1 *L. chagasi* antigen (similar to a *T. cruzi*flagellar protein) in BCG and the KMP-11 (kinetoplastid) antigen in attenuated tachyzoites of *Toxoplasma gondii*. Examples of vaccines based on virus are Vaccinia virus expressing the G46/M-2/PSA-2 promastigote surface protein which protects against *L. amazonensis*; or Vaccinia expressing the *L infantum* LACK antigen (parasite analogue to the receptor for activated mammalian kinase C) which in prime boost vaccination, protects mice against *L. major*[62] and dogs against *L.*

infantum infection. Recombinant antigen can be derived as purified proteins, as the naked DNA encoding them or as bacteria manufacturing the proteins in situ. Manipulations now allow targeting of the antigen to specific locations or to particular antigen-presenting cells, such as dendritic cells or langerhans cells, which are considered essential for the initiation of primary T cell responses, injection of bacteria or naked DNA may have the added advantage of providing an adjuvant effect, which may “activate” or “licence” these antigen-presenting cells[63].

The first recombinant antigen used to vaccinate against leishmaniasis was leishmaniolysin or gp63. This is an M,65,000 memebrane protease present in promastigotes of all species. gp63 is one of the parasite receptor s for host macrophages and parasite mutanys lacking the protein are avirulent[64]. It is unfortunate that in humans and animal models the T cell response to gp63 have been variable. However when detected, they appeared to be of the Th1 type[65]. Overall gp63 is still considered a promising vaccine candidate. A second vaccine candidate tested in animal models is a membrane antigen of unknown function, gp46/M2 or parasite surface antigen 2(PSA-2)[66]. As with gp63, PSA-2 belongs to a multigenic family expressed in all *leishmania* species except *L. brazilliensis*. Its presence in most species makes PSA-2 an attractive candidate for a pan-*leishmania* vaccine. PSA-2 protect against *L. major* as well as *L. Mexicana* when administered as purified protein or expressed in vaccinia virus. Recombinant DNA derived PSA-2 protein was variable in its ability to confer protection, while the protein derived from the yeast pichia pastoris provided good protection. The leishmanial eukaryotic ribosomal protein (LeIF), a homologue of the ribosomal protein cIF4A is being considered as a vaccine candidate based on its ability to induce Th1 type cytokines in humans[67]. A similarly conserved antigen the *leishmania* homologue of the receptor for activated C kinase (LACK) which is expressed by both promastigotes and amastigotes has been shown to protect mice from infection, in particular when administered with IL-12 as an adjuvants (45,100). Interestingly, LACK is also the major target for Th 2 responses in susceptible BALB/C mice and BALB/C mice made tolerant to LACK are resistant to infection. Several other vaccine candidates identified in the last few years are in the process of being characterized. Some are amastigote specific, such as A2, P4 and P8 of *L. Mexicana* pifanoi. Another vaccine candidate is a flagellar antigen Icr 1, from *L.donovani cahgasi*[68]

Vaccines based on purified leishmania antigens :

A further approach to second-generation vaccines includes the purified *Leishmania* sub fractions. Proteins or lipophosphoglycan have been used to assess their immunogenicity, but because of difficulties in their mass production, they never advanced to Phase IIa or Phase III trials. In the first Phase III trial with a second-generation dog vaccine, Dunan et al. in 1989, using a semi-purified lyophilized protein preparation from *L. infantum* (94—67 kDa), paradoxically achieved a significantly higher rate of infection in the vaccinate group than in the control group. This vaccine then, while effective in murine models, did not induce protectionagainst canine kala-azar in the field. The other second-generation vaccine, *Li*ESAp, composed of the 54 kDa excreted protein of *L. infantum* plus MDP, protected dogs in a kennel assay against *L. infantum* infection. Parasites were detected in the bone marrow of 3/3 placebo treated controls, while they were absent in 0/3 vaccinated dogs[69]. A double-blind randomized trial was further performed with *Li*ESAp + MDP in naturally exposed dogs in Southern France[69]. After 2 years, the incidence of infection was 0.61% (1/165) in vaccinees versus 6.86% (12/175) in control dogs, corresponding to a 92% VE. In any dog showing clinical and/or serological evidence, infection was confirmed by the presence of parasites in bone marrow aspirates cultured in NNN media and also by PCR analysis[69]. In contrast to results of the FML-saponin vaccine[70], *Li*ESAp vaccine induced protection against infection[69], but not against severe disease or death by VL. The FML-vaccine is considered a second-generation vaccine candidate and it was featured at the fourth Meeting on Second-Generation *Leishmania* vaccines held in M’erida in May 2001. When used at double adjuvant concentration, it is also immunotherapeutic for dogs naturally[70] or experimentally infected[71] with *Leishmania chagasi*. The sustained proportions of CD4 and CD21 lymphocyte levels in blood of vaccinated animals[70,71] indicate that the FML-vaccine reduces dog infectivity to sand flies. The adjuvants used in the FML-vaccine are the QS21 and aldehyde-containing deacylated saponins of *Quillaja saponaria*[72]. In 2004, the FML-vaccine became the first second-generation vaccine licensed for prophylactic veterinary use, under the name of LeishmuneR[73]. Recently, the mechanism of action of the QS21 saponin was elucidated. QS21 contains two carbohydrate chains

attached to a triterpene nucleus C3 and C28, besides a hydrophobic moiety which is acylated to a C28 sugar attached residue. The QS21 hydrophobic moiety is related to the induction of the CTL CD8+ protective lymphocyte response, while the aldehyde group present in triterpene C4 is involved in direct T lymphocyte stimulation, mimicking the B7-1 co-stimulatory molecule to induce the TH1 protective response. Preliminary results suggest that protection induced by the FML-QS21 vaccine is also related to the activation of a bradikinin mediated inflammatory response at the site of injection, which stimulates immature dendritic cells through their B1R and B2R surface receptors thereby triggering a Th 1 response against *Leishmania chagasi*[74].

Third generation vaccines

Compared to recombinant protein vaccines, DNA vaccines are much more stable and have the advantage of their low cost of production, no need of cold chain for distribution, and flexibility of combining multiple genes in a simple construct. The mechanism by which DNA vaccination generates potent immune responses appears to be through the activation of innate immune responses by the non-methylated CpG sequences of bacteria and to the intense replication within the host, leading to the expression of the recombinant proteins for longer periods.The most-studied antigens were those previously assayed as recombinant proteins[16,75]. Most of them were tested as single vaccines[76], and some, as combination of genes[75,77] or as heterologous prime-boost (HPB), which involves an injection of the DNA vaccine followed by an injection of the recombinant protein[15] or a Vaccinia virus expressing the recombinant protein. Adjuvants were added to formulations in only two studies[15] (Table 3). Protection was observed in vaccines using all the tested plasmids[16,76], with the exception of pMOK in a dog assay, and of pcDNA3 in a mice assay. Most trials were performed in mice against CL and VL[78], and some in the hamster against VL[79] and in dogs against CVL. All the studies involved artificial challenge (Phase I—IIa) with agents of the New and Old World leishmaniasis. The most striking characteristic of the DNA vaccine studies is that about a half of them used for challenge non-virulent (NV) *Leishmania* strains, grown in liquid culture media[78] and at a low number of parasites[62], while the rest used virulent [V] parasites isolated from infected animals[16,79]. This heterogeneity of protocols determined a high variation of percents of reduction of parasite load, with a mean average of 59.24% (IC 95% 47.75—70.73). The LACK, LeIF, TSA, LmSTI1, H1, CpA + CpB, KMP11 and NH36 are the most promising candidates that may find a place in the forth coming years (Table 1), since they have already been tested in more animal models. In mice, LACK DNA induced a TH1 response that protected against infection by *L. major*, but not *L.donovani*. Even truncated portions of the LACK gene and PSA2 gene were superior to GP63 and p20 against *L. major* infection, and immunization with HPB-LACK protected mice against VL and dogs from CVL. The immunization resulted in an increase in IFN-γ and IL-12 expression, lymphocyte proliferative response, IgG2 to IgG1 ratio while it led to decreases in clinicalsymptoms, number of parasites in target tissues, and IL-4 expression. Vaccination of mice either with the TSA or the LmSTI1 DNA vaccines, or with both as a tandem digene construct[80], protected against CL through a CD4 + TH1 response. The digene and the TSA gene were the most protective, with the latter involving a CD8+ response[80]. Injection of a mixture of four histone plasmids (H2A, H2B,H3 and H4) in Balb/c mice also protected against *L. major* infection through a Th1 response. While plasmid CPa and CPb on their own did not, the combination of both conferred protection on mice against *L. major*. Vaccination with 200 _g of KMP11 protected hamsters against VL through a mixed cytokine Th1/Th2 response[79], while a cocktail of plasmid DNA encoding KMPII, TRYP, LACK, and GP63 (200 _g of each plasmid) did not protect dogs against *L. infantum* virulent challenge. The NH36 DNA vaccine protected mice against infection by *L. chagasi*, *L. mexicana*[16], and *L. amazonensis*, indicating its potential usefulness in a bivalent immunoprophylactic vaccine for the control of both endemics. The comparison of the efficacies achieved by the different generations of vaccines against leishmaniasis is summarized in Table 2. While the first-generation vaccines, with or without adjuvant, display a relatively low VE, the second-generation vaccines with native antigens show a significant increase in VE. The VE results derived from these two groups of vaccines are very robust, since they arise from field trials with exposure to natural challenge in the target species (human and dogs). On the other hand, while the protection induced by the recombinant protein second-generation vaccines are slightly greater, neither the recombinant nor the DNA vaccines are significantly different from the first-generation ones. It is worth noting that the

results of these last two groups are calculated based on reduction of parasite load achieved mainly in mice laboratory studies.

Table 1: Promising candidates for third generation vaccine

Candidate antigen	Tested animal model
LACK	Mice and Dogs
LelF, TSA, LmSTI1	Mice, Monkey, Human
H1	Mice, Monkey
Cpa + CPb	Mice, Dogs
KMP11	Mice, Hamsters, Dogs
NH36	Mice, Dogs

Table 2: Efficacy of vaccines for leishmaniasis developed so far

Generation	Adjuvants	Phase	Average protection	Evaluation	Model
1st	Yes/No	III	54.38	VE	Human, Dog
2nd native	Yes	III	82.66	VE	Dog
2nd recombinant	Yes	IIa	68.02	Parasite load	Mice, Monkey, Dog
3rd	No	IIa	59.24	Parasite load	Mice, Dog

Chemotherapy

Almost all forms of leishmaniasis should be treated. Drug efficacy might be affected by the parasite species, gender and age, body distribution of the disease, immunological status of the host and mode of treatment. Most patients respond to treatment, but in certain cases of *L. tropica, L. aethiopica, L. braziliensis and L. amazonensis*, conventional treatment is only partially effective[81]. The pentavalent antimonial compounds, sodium stibogluconate (pentostam) and N-methyl meglumine antimonite (glucantine), are considered the drugs of choice against all forms of the disease, generally with mild side effects. Alternative therapies include pentamidine and amphotericin B, which are relatively toxic agents and are sometimes associated with mild or severe side effects. Orally administered agents i.e ketoconazole, itraconazole, fluconazole and allopurinol, either alone or in combination, have shown variable levels of efficacy[81]. Miltefosine, a newly developed drug taken orally, has yielded encouraging results against VL and Old & New World CL[82,83]. Physical treatment, including irradiation, cauterization, freezeing by liquid nitrogen, infra red and photodynamic climatotherapy, has also been used to treat CL[81]. Topical application with AmB, ethanolic lipid AmB, and various formulated paromomycin (aminosidine) ointments were also effective against CL.

Resistance to chemotherapeutic treatment

Parasite drug resistance was demonstrated with most of the available drugs against the disease. Only a small number of effective drugs against the disease are available and most of the parasites develop drug resistancy. In Bihar, India, 40-60% of patients suffering from VL did not respond to pentavalent antimony treatment[84]. Similar results, with 25% unresponsiveness, were recorded with pentamidine. Lack of response to AmB is uncommon, every though resistance to this drug was observed in *L. infantum*/human immunodeficiency virus-infected cases. Resistance of *L. donovani* promastigotes to miltefosine was shown in-vitro, and resistance of both *L. major* and *L. tropica* promastigotes to paromomycin was induced in-vitro by repeated exposure of the parasites while gradually increasing the drug concentrations.

Immunochemotherapy

Immunochemotherapy have been used to accelerate the specific immune response in immunologically responding and non-responding patients. The concept was to selectively induce Th1 responses that are considered essential for resistance to leishmaniasis. Various efforts were done by reasearcher to completely cure the leishmaniasis.

In a study using guinea pigs with bith ears infected with *L. enriettii*, a topical treatment with aromomycin/methylbenzethoniumchloride ointment on one ear also caused a delayed therapeutic effect on the untreated ear. This phenomenon was further demonstrated in humans suffering from CL caused by *L.major*.

Treating one lesion affected the healing of other untreated lesion in the same patient. It was suggested that local treatment causing damage to the parasites is followed by the release of leishmanial antigenic constituents, which evoke an immune response that leads to the regression of lesion in distant sites of the body. Indeed, both purified and recombinant specific antigens and DNA encoding antigens have been applied in the development of a protective vaccine against leishmaniasis[17].

Chemotherapy of canine VL, caused by *L.chagasi, L.donovani* and *L.infantum* is only partially effective and immunotherapy is a new approach to control the disease[71]. Approximately 100% protection against canine VL was achieved in dogs vaccinated with LiESAp vaccine (a 54 kDa excreated protein of *L.infantum*) combined with muramyl dipeptide[85]. However while crude parasite extracts together with Glucantime were only partially effective, purified antigen (LiF2,L.infantum-derived fraction 2) combined with Glucantime cured all dogs within 6 months. The saponin-enriched Leishmune vaccine given alone was found only moderately effective against *L.chagasi*[71], but the polyprotein recombinant vaccine Leish-110f, together with the adjuvant MPL-SE and meglumine antimonite induced elevated cellular immune responses, improved the clinical symptoms and reduced the mortality rate in symptomatic dogs[86].

A combination of antileishmanial chemotherapeutic agents together with various immunomodulator (MDP13,IFN-γ, IL-12) accelerated treatment efficacy in ACL, MCL and DCL patients, caused by various Leishmania strains. Either a moderate or a synergestic effect of IFN-γ plus pentavalent antimonal compounds was demonstrated against intracellular L.donovani amastigotes in-vitro and against VL in mice and humans. In murine leishmaniasis, recombinant Th1 stimulating cytokine IL-12 given alone or combined with either sodium stibogluconate or paromomycin cured mice from *L.major* infection. Treatment with recombinant IFN-γ and Th1 stimulating cytokine IL-12 also has a positive effect. IL-18, a potent inducer of IFN-γ production by T cells, did not influence the therapeutic activity against *L.donovani* infection. Human DCL and MCL respond very poorly to convential therapy and chemotherapy generally produces only transitory remission of the disease. Immunotherapy using antimonials combined with IFN-γ was only marginally effective. However GM-CSF14 plus either a mixture of *L.major* antigens (LmSI1+LeIf+HSP 83)[87] or meglumine antimonite [Almeida et al.,2005] was reported as being highly effective in treating American CL and MCL. Recently a combination of Z-100, a polysaccharide obtained from Mycobacterium tuberculosis combined with pentavalent antimonial, was found effective against *L.amazonensis in-vitro*.

In experimental VL neutralizing IL-10 or blocking its receptor was preferable for the induction of Th1 cytokine activity, leading to IFN-γ secretion, granuloma formation, macrophage activation and parasite killing, which were futher increased in combination with pentavalent antimonials[Murray et al.,2005]. However, suppression of other cytokines, including receptor fusion antagonists of IL-13, IL-14 and TGFβ, inhibited parasite replication but only marginally affected parasite killing without the induction of a synergistic effect with pentavalent antimonials[88].

A combination of topical monomycin and injectable leukinferon (a complex of IL-1, TNF-α, IFN-γ and macrophage migration inhibitory factorcytokines) was successful in treating zoonotic CL in Uzbekistan. Imiquimod (IMQ, 3M pharmaceuticals USA) a low molecular weight imidazoquinoline, is a novel immune response activating agent, found to induce IFN-γ, TNF,IL-1β,IL-1α,IL-6,IL-10,IL-1 receptor antagonist GM-CSF and granulocyte CSF,IMQ applied topically has been shown to act synergistically with meglumine antimonite in the treatment of CL in experimentally infected mice. This combination was also found highly effective in the treatment of CL caused by *L.peruviana* in Peru, but less so against CL caused by *L.tropica* in Iran. No synergestic effect was observed with an ointment comprising Imiquimod combined with Leshcutan (15%paromomycin + 12%methylbenzethonium chloride) in the treatment of BALB/C mice infected with *L.major*.

Liposomal Amphotericin B is considered superior to free AmB in the treatment of visceral leishmaniasis. L-AmB combined with rHuGM-CSF or meglumine antimonite was found effective against VL in AIDS patients and against CL caused by *L.braziliensis* in Brazil. DC-based immunotherapy combined with antimony based chemotherapy was very effective against murine VL. While three weekly injections of *L.donovani* soluble antigen pulsed DCs into mice infected with *L.donovani* only reduced the number of spleen and liver amastogotes, treatment with sodium

stibogluconate combined with antigen plused DCs resulted in a complete deletion of the parasites.

CONCLUSION

With this study we conclude that immunotherapy and chemotherapy separately is well tolerated in humans. Various trials were taken using animal models in Europe and other developed countries, but the lack of scientists in regulatory agencies of underdeveloped countries. However as judged by the various studies, we suspect that the combination therapy may have accelerated the early parasitologic response. If this effect is borne out in a larger controlled trial recently initiated in India using IFN-γ administered daily, combination immunochemotherapy may permit in reduction in the duration and/or dose of antileishmanial drugs required to achieve a satisfactory clinical and parasitoligic response. Hence chemotherapy including various antileishmanial drugs in combination with immunotherapy using various vaccines can completely cure leishmaniasis.

REFERENCES

1. WHO, 2000, fact sheet no 116, WHO, Geneva.
2. Doron Rosenzweig, Derek Smith, Fred Opperdoes, Shay Stern, Robert W.,Olafsan and Zilberstein(2008), Retooling leishmania metabolism from sandfly gut to human macrophage, the FASEB Journal,22,590-602.
3. WHO conference bulletein. 19th june 2009.
4. WHO-TDR, The TDR fifteenth program report, research progress 1999-2000, new and improved tools, 2003, www.who.int/tdr/research/progress9900/tools/udr.htm.
5. Alvar J., Canavate C., Gutierrez-solar B, Jimenez M., Laguna F ., Lopez-Uelez R., Leishmania and human immunodeficiency virus coinfection : the first 10 years, clin microbial rev.1997;10:298-319.
6. Solbach W, Laskay T. The host response to leishmania infection. Adv. Immunol.(2000).74:275.
7. Gavron D, Saul A. pioneer of tropical medicine rehovot, Israel. Balaban publishers.1997;ISBN.O-86689-045-9.
8. Kamesipour A., Rafati S., Davondi N., Maboudi F. leishmaniasis vaccine candidates for development : a global overview Ind J Med Res.2006;123:423-38.
9. Modabber F. Vaccine against leishmaniasis. Ann Trop Med Parasitol.1995;89:83-8.
10. Yang DM, Fairweather N, Button LL, McMaster WR, Kahl LP, Liew FY. Oral Salmonella typhimurium (AroA-) vaccine expressing a major leishmanial surface protein (gp63) preferentially induces T helper 1 cells and protective immunity against leishmaniasis. J Immunol 1990;145:2281—5.
11. Skeiky YAW, Guderian JA, Benson DR, Bacelar, Carvilha EM, Kubin M. A recombinant leishmania antigen that stimulates human peripheral blood mononuclear cells to express a th1 type cytokine profile and to produce interleukin12. J Exp Med.1995;181:1527-37.
12. Jardim A,Tolson DL, Turco SJ, Pearson TW, Olafson RW.The leishmania donovani lipophosphoglycan T lymphocyte reactive component is a tightly associated protein complex. J Immunol.1991;147:3538-44.
13. Rachamin N, Jaffe CL. Pure protein from leishmania donovani protects mice against both cutaneous and visceral leishmaniasis. J Immunol.1993;150:2322-31.
14. XuD, Liew FY. Genetic vaccination against leishmaniasis. Vaccine.1994;12:1534-6.
15. Rafati S, Nakhaee A, Taheri T, Taslimi Y, Darabi H, Eravani D. Protective vaccination against canine visceral leishmaniasis using a combination of DNA and protein immunisation with cystein proteinases type I and type II of leishmania infantum. Vaccine.2005;23:3716-25.
16. Aguilar Bel, Zardo RS, Paraguai de Souza E, Borja-Cabrera GP, Rosado-Vallado M, Mut-Martin M. Cross protective efficacy of a prophylactic leishmania donovani DNAvaccine against visceral and cutaneous murine leishmaniasis. Infect Immun.2005;73:812-9.
17. Palatnik-de-sousa CB, Vaccine for leishmaniasis in the forecoming 25 years. Vaccine.2008;26:1709-24.
18. Joseph EL. Current status and perspectives of the immunotherapy of leishmaniasis. IMAJ.(2009).11:623-628.

19. Mauel J, Behin R. Immunity : clinical and experimental. The leishmaniases, vol 2, eds Peters W and Killick-Kendric R, Academic press, London(1987), 731-791.
20. Liew FY, O'Donell CA. Immunology of leishmaniasis. Adv. Parasitol.,32:161-259.
21. Scharton-Kersten T, Afonso LC, Wysocka M, Trinchieri G, Scott P. IL-12 is required for natural killer all activation and subsequent T helper 1 cell development in experimental leishmaniasis. J Immunol.(1995).154:5320.
22. Diefenbach A, Schindler H, Donhauser N. Type 1 interferon (IFN-α/β) and type 2 nitric oxide synthase regulate the innate immune response to protozoan parasite. Immunity.(1998).8:77.
23. Melby PC, Anstead GM. Immune responses to protozoa. In : Clinical immunology. Edited by Rich RR, Fleisher TA, Shearer WT, Kotzin BL, Schroder Jr HW, 2nd edition, Mosby International Limited.(2001).29:1-29.13.
24. Reiner SL and Locksley MR. The regulation of immunity to Leishmania major. Annu. Rev. Immunol.(1995).13:151.
25. Tsagozis P, Karagouni E, Dostsika E. CD8(+) T cells with parasite-specific cytotoxic activity and a TCL profile of cytokine and chemokine secretion develop in experimental leishmaniasis. Parasite Immunol.(2003).25(11-12):569-579.
26. Stenger S, Thuring H, Rollinghoff M, Bogdan C. Tissue expression of inducible nitric oxide synthase is closely associated with resistance to leishmania major. J. Exp. Med.(1994).180:783.
27. Noben-trauth N, Paul WE, Sacks DL. IL-4 and IL-4 receptor deficient BALB/C mice reveal differences in susdeptibility to leishmania major parasite substrains. J. Immunol., 162-6132.
28. Goto H, Lindoso JAL. Immunity and immunosuppression in experimental visceral leishmaniasis. Braz Med J Biol Res.(2004).37:615-623.
29. Rodrigues Jr V, Silva JS, Campos-Neto A. Transforming growth-factor β and immunosupression in experimental visceral leishmaniasis. Infect Immun.(1998).66:1233-6.
30. Gomes NA, Barreto-de-souza V, Wilson ME, Dos Reis GA. Unresponsiveness CD4+ T lymphocytes from leishmania chagasi infected mice increases cytokine production and mediate parasite killing after blockade of B7-1, CTLA-4 molecular pathway. J Inf Dis.(1998).178:1847-51.
31. Anam K, Afrin F, Banerjee D, Pramanik N, Guha SK et al. Differential decline in leishmania membrane antigen specific immunoglobulin G (IgG), IgM, IgE, and IgG subclass antibodies in Indian Kala-azar patients after chemotherapy. Infect Immun(1999).67:6663-9.
32. Atta AM, D'Oliveira, Correa J, Atta ML, Almeida RP, Carvalho EM. Antileishmanial IgE antibodies : a marker of active disease in visceral leishmaniasis. Am J Trop Med Hyg.(1998).59:426-30.
33. Da-Matta VL, Hoshino-Shimizu S, Dietze R, Corbett CE. Detection of specific antibody isotypes and subtypes before and after treatment of American visceral leishmaniasis. J Clin Lab Anal(2000).14:5-12.
34. 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.
35. Ravindran R, Anam K, Bairagi BC, Saha B, Pramanik N, Guha SK et al. Characterization of immunoglobulin G and its sub-class response to Inadin Kala-azar infection before and after chemotherapy. Infect Immun.(2004).72:863-70.
36. Handman E. Leishmaniasis : current status of vaccine development. Clinical microbiology Reviews.(2001).14(2):229-243.
37. Greenblatt CL. The present and future of vaccination for cutaneous leishmaniasis. Clin.Biol.Rs.(1980).47:259-285.
38. Kellina OI. Problems and current lines in investigations on the epidemiology of leishmanisis and its control in the USSR. Bull Soc Pathol Exot.(1981).74:306-318.

39. Kellina OI. Changes in virulence of strains of leishmania tropica major. Med Parasitol.(1965).
40. Serebryakov VA, Karakhodznaeva SKH, Dzhumaev MD. Effect of leishmanial vaccinations on the dynamics of immunity to diphtheria in conditions of secondary revaccination with adsorbed pertusis-diphteria-tetanus vaccine. Med Parasitol Mosk.(1972).41:303-9.
41. Berberian DA. Cutaneous leishmaniasis (oriental sore). IV. Vaccination against oriental sore with suspension of killed leishmania tropica. Arch. Dermatol. Syphilol.(1944).35(8):561-65.
42. Pessoa SB, Pestana BR. Enseaio sobre vacinacao preventive na leishmaniose tegumentar Americana com germens mortos. Arq Hig Saude Publ.(1941).6:141-7.
43. Pessoa SB. Segunda nota sobre a vacinacao preventive na leishmaniose tegumentar Americana com leptomones mortas. Rev Med.(1941).9:106.
44. Mayrink W, Antunes CM, Da Costa CA, Melo MN, Dias M, Michalick MS, Magalhaes PA, De Oliviera Lima A, Williams P. Further trials of a vaccine against American cutaneous leishmaniais. Trans R Soc Trop Med Hyg.(1986). P. 80.
45. Mayrink W, DaCosta CA, Magathaes PA, Melo MN, Dias M, Lim AO, Michalick MS, Williams P. A field trial of a vaccine against American dermal leishmaniasis. Trans R Soc Trop Med Hyg(1979).73:385-387.
46. Alexander J. A radioattnuated leishmania major vaccine markedly increases the resistance to CBA mice to subsequent infection with leishmania Mexicana. Trans R Soc Trop Med Hyg.(1982).76:646-649.
47. Sokolova AN. Preventive vaccination with the living parasites of cutaneous leishmaniasis. Turkman. Cutan-Va=enereol. Inst. Ashkhabad.(1940).11-44.
48. Larmukhamedov MA. On the question of counter indications to vaccination to cutaneous leishmaniasis. Med. Parasitol.(Moskow).(1971).50:549-552.
49. Velez ID, Gilchrist K, Arbelaez MP, Rojas CA, Puerta JA, Antunes CM et al. Failure of a killed leishmania amazonensis vaccine against American cutaneous leishmaniasis in Colombia. Trans R Soc Trop Med Hyg. 2005;99:593-8.
50. Armijos RX, Weigel MM, Calvopina M, Hidalgo A, Cevallos W, Correa J. Safety, immunogenicity and efficacy of an autoclaved leishmania amazonensis vaccine plus BCG adjuvant against new world cutaneous leishmaniasis. Vaccine.2004;22:1320-6.
51. Mendonca SCF, DaLuca PM, Mayrink W, Rstom TG, Conceicao-silva F, De-cruz AM, Bertho AL, DaCosta CA, Genaro O, Toledo CP, Coutinho SG. Charaterization of human T-lympocytes mediated immune responses induced by a vaccine against American tegumentary leishmaniasis. Am. J. Trop. Med. Hyg.(1995).53:195-201.
52. Satti IN, Osman HY, Daifalla NS, Younis SA, Zijlstra EE et al. Immunogenicity and safety of autoclaved leishmania major plus BCG vaccine in healthy Sudanese volunteers. Vaccine 2001;19:2100-6.
53. Sharifi I, Fekri AR, Aflatonian MR, Khamesipour A, Zicker F, Mousavi MR et al. Randomised vaccine trial of single dose of killed leishmania major plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. Lancet.1998; 351(9115):1540-3.
54. Momeni AZ, Jalayer T, Emamjomeh M, Khamesipour A, Zicker F, Ghassemi RL, et al. A randomised double blind controlled trial of a killed L. major vaccine plus BCG against zoonotic cutaneous leishmaniasis in Iran. Vaccine.1999;17:466-72.
55. Mayrink W, Botelho ACC, Magalhaes PA, Batista SM, Lima AO, Genaro O et al. Immunotherapia, imunoquimioterapia e quimioterapia no tratamento da leishmaniose tegumentar Americana. Rev Soc Bras Med Trop 2006;39:14-21.
56. Alexander J, Coombs GH, Mottram JC. Leishmania Mexicana cysteine proteinase-deficient mutants have attenuated virulence for mice and potentiate a TH1 response. J Immunol. 1998;161:6794—801.
57. Papadopoulou B, Roy G, Breton M, Kunding C, Dumas C, Fillion I, et al. Reduced infectivity of a Leishmania donovani biopterin transporter genetic mutant and its use as an attenuated

strain for vaccination. Infect Immun 2002;70:62—8.
58. Requena JM, Iborra S, Carri'on J, Alonso C, Soto M. Recent advances in vaccines for leishmanasis. Exp Opin 2004;4:1505—17.
59. Titus RG, Bishop JV, Mejia JS. The immunomodulatory factors of arthropod saliva and the potential for theses factors to serve as vaccine targets to prevent pathogen transmission. Parasite Immunol 2006;28:131—41.
60. Metz DP, Bottomly K. Function and regulation of memory CD4 T cells. Immunol. Res 1999;19:127-141.
61. Xu D, McSorley SJ, Chatfield SN, Dougan G, Liew FY. Protection against Leishmania major infection in genetically susceptible BALB/c mice by gp63 delivered orally in attenuated. Salmonella typhimurium (AroA- AroD-). Immunology 1995;85:1—7.
62. Gonzalo RM, del Real G, Rodriguez JR, Rodriguez D, Heljasvaara R, Lucas P, et al. A heterologous prime-boost regime using DNA and recombinant vaccinia virus expressing the Leishmania infantum P36/LACK antigen protects BALB/c mice from cutaneous leishmaniasis. Vaccine 2002 Jan 15;20(7—8):1226—31.
63. Matzinger P. An innate sense of danger semin. Immunol.1998;10:399-415.
64. Chang KP, Chaudhury G, Fong D. Molecular determinants of leishmania virulence. Ann Rev Microbiol.1990;44:499-529.
65. Russo DM, Burns JMJ, Carvalho EM, Armitage RJ, Grabstein KH, Button LL, McMaster WR, Reed SG. Human T cell responses to gp63, a surface antigen of leishmania. J Immunol.1991;147:3575-3580.
66. Handman E, Symons FM, Baldwin TM, Curtis JM, Scheerlink JPY. Protective vaccination with promastigote surface antigen 2 from leishmania major is mediated by Th1 type immune response. Infect Immunol.1995.63:4261-4267.
67. Skeiky YA, Kennedy M, Kaufman D, Borges MM, Guderian JA, Scholler JK, Ovendale PJ, Picha KS, Morrissey PJ, Grabstein KH, Campos-Neto A, Reed SG. LeIF : a recombinant leishmania protein that induces an IL-12 mediated Th1 cytokine profile. J Immunol.1998;161:6171-6179.
68. Streit JA, Recker TJ, Donelson JE, Wilson ME. BCG expressing LCR1 of Leishmania chagasi induces protective immunity in susceptible mice. Exp Parasitol 2000;94:33—41.
69. Lemesre JL, Holzmuller P, Gonc¸alves 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.
70. Borja-Cabrera GP, Correia Pontes NN, da Silva VO, Paraguai de Souza E, Santos WR, Gomes EM, et al. Long lasting protection against canine kala-azar using the FML-QuilA saponin vaccine in an endemic area of Brazil (S˜ao Gonc¸alo do Amarante).Vaccine.2002;20:3277—84.
71. Santos FN, Borja-Cabera GP, Miyashro LM et al. Immunotherapy against experimental canine visceral leishmaniasis with the saponin enriched leishmania vaccine. Vaccine.2007;25:6176-90.
72. 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(19):2470—9.
73. Nogueira FS, Moreira MAB, Borja Cabrera GP, Santos FN, Menz I, Parra LE, et al. LeishmuneR vaccine blocks the transmission of canine visceral leishmaniasis. Absence of Leishmania parasites in blood, skin and lymph nodes of vaccinated exposed dogs. Vaccine 2005;23:4805—10.
74. Nico D, Souza LOP, de Almeida LN, Monteiro ACS, Scharfstein J Palatnik-de-Sousa CB. Kinins generated upon vaccination with the QS21 saponin-containing LeishmuneR vaccine are partially responsible for induction of protective immunity against visceral leishmaniasis. Vaccine Congress. Celebrating 25 years of publication. 9—11 December, Amsterdam, the Netherlands.2007.
75. Iborra S, Soto M, Carrion J, Alonso C, Requena JM. Vaccination with a plasmid DNA cocktail encoding the nucleosomal histones of Leishmania

confers protection against murine cutaneous leishmaniosis. Vaccine 2004 Sep 28;22(29—30):3865—76.
76. Gamboa-Le'on R, Paraguai de Souza E, Borja Cabrera GP, Santos FN, Miyashiro LM, Pinheiro RO, et al. Immunotherapy against visceral leishmaniasis with the nucleoside hydrolase- DNA vaccine of L. donovani. Vaccine 2006;24:4863—73.
77. Campbell K, Diao H, Ji J, Soong L. DNA immunization with the gene encoding P4 nuclease of Leishmania amazonensis protects mice against cutaneous Leishmaniasis. Infect Immun 2003;71:6270—8.
78. Liew FY, Xu D. Protection against leishmaniasis by injection of DNA encoding a major surface glycoprotein, gp63, of L. major. Immunology 1995;84:173—6.
79. Basu R, Bhaumik S, Basu JM, Naskar K, De T, Roy S. Kinetoplastid membrane protein-11 DNA vaccination induces complete protection against both pentavalent antimonial-sensitive and -resistant strains of Leishmania donovani that correlates with inducible nitric oxide synthase activity and IL-4 generation: evidence for mixed Th1- and Th2-like responses in visceral leishmaniasis. J Immunol 2005;174:7160—71.
80. Campos-Neto A, Webb JR, Greeson K, Coler RN, Skeiky YA, Reed SG. Vaccination with plasmid DNA encoding TSA/LmSTI1 leishmanial fusion proteins confers protection against Leishmania major infection in susceptible BALB/c mice. Infect Immun 2002;70:2828—36.
81. Croft ST, Yardley V. Chemotherapy of leishmaniasis. Curr Pharmaceut Des.2002;8:319-42.
82. Soto J, Rea J, Balderrama M at al. Efficacy of miltefosine for Bolivian leishmaniasis. Am J Trop Med Hyg.2008;78:210-11.
83. Sundar S, Olliaro PL. Miltefosine in the treatment of leishmaniasis : clinical evidence for informed clinical risk management. The Clin Risk Manag.2007;3:733-40.
84. Jha TK. Drug unresponsiveness and combined therapy for Kala-azar. Indian J Med Res.2006;123:389-98.
85. Lemesre JL, Holzmuller P, Goncalves RB et al. Long lasting protection against visceral leishmaniasis using the LiESAp-MDP vaccine in endemic areas of France double blind randomised efficacy field trial. Vaccine.2007;25:4223-34.
86. Miret I, Nascimento E, Sampaio Wet al. Evaluation of an immunochemotherapy protocol constituted N-methyl meglumine antimonite Leish-110f + MPL-SE vaccine to treat canine visceral leishmaniasis. Vaccine.2008;26:1585-94.
87. Badaro R, Lobo I, Munos A et al. Immunotherapy for drug refractory mucosal leishmaniasis. J Infect Dis.2006;194:1151-9.
88. Murray HW, Flanders KC, Donaldson DD et al. Antagonizing deactivating cytokines to anhance host defence and chemotherapy in experimental visceral leishmaniasis. Inf immune.2005;73:3903-11.