ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 02, pp.45973-45977, February, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

CURRENT SCENARIO OF VACCINE DEVELOPMENT AGAINST A NEGLECTED DISEASE: VISCERAL LEISHMANIASIS

***Dr. Sabiha Imran**

Department of Biotechnology, Faculty of Engineering and Technology, ManavRachna International University, Faridabad, Haryana, INDIA

ARTICLE INFO

Article History:
Received 18th November, 2016
Received in revised form
14th December, 2016
Accepted 20th January, 2017
Published online 28th February, 2017

Key words:

Visceral leishmaniasis,
Recombinant vaccines,
DNA vaccines,
Mutant vaccines,
Synthetic peptide vaccines.

ABSTRACT

Visceral leishmaniasis (VL) or kala-azar, a vector-borne protozoan disease, shows endemicity in larger areas of the Asia, Africa, South America, and the Mediterranean basin. WHO report suggested that an annual incidence of VL is nearly 200,000 to 400,000 cases, resulting in 20,000 to 30,000 deaths per year. Treatment with available anti-leishmanial drugs are not cost effective, with varied efficacies and higher relapse rate, which poses a major challenge to current kala-azar control program in Indian subcontinent. Therefore, a vaccine against VL is imperative and knowing the fact that recovered individuals developed lifelong immunity against re-infection, it is feasible. Vaccine development program, though time taking, has recently gained momentum with the emergence of omic era, i.e., from genomics to immunomics. Classical as well as molecular methodologies have been overtaken with alternative strategies wherein proteomics based knowledge combined with computational techniques (immunoinformatics) speed up the identification and detailed characterization of new antigens for potential vaccine candidates. This may eventually help in the designing of polyvalent synthetic and recombinant chimeric vaccines as an effective intervention measures to control the disease in endemic areas. This review focuses on such newer approaches being utilized for vaccine development against VL.

Citation: Dr. Sabiha Imran, 2017. "Current scenario of vaccine development against a neglected disease: visceral leishmaniasis", *International Journal of Current Research*, 09, (02), 45973-45977.

INTRODUCTION

Visceral leishmaniasis (VL), synonymously known as kala-azar, is caused by obligate intra-macrophage protozoan parasite and is characterized by both diversity and complexity (Chappuis *et al*., 2007). The disease is prevalent in larger areas of tropical, subtropical, and the Mediterranean countries. As per WHO report, nearly 200,000 to 400,000 new cases of VL (with an average duration of several months to more than one year) occur annually with 20,000 to 30,000 deaths per year which is lesser than by malaria among parasitic diseases, although its exact impact has been underestimated as an exact number of cases were never recorded. Ninety percent of the VL cases occur in Bangladesh, Brazil, India, Nepal, and Sudan. In India, 80% VL cases were only from the state of Bihar (Hasker E *et al*., 2012). The arthropod vector – female phlebotomine sandflies, nocturnal, and telmophagous, are responsible for the transmittance of the disease. Two species – Leishmaniadonovanidonovani (in East Africa and the Indian subcontinent) and L. donovaniinfantum (in the Mediterranean region of Europe, North Africa, and Latin America) are the main causative organisms for VL (Lukes, 2007). The parasite bears two distinct life forms: promastigote, a flagellar form, found in the gut of the vector, which is inoculated into the dermis where it is internalized by dendritic cells and the macrophages and eventually is transformed into an aflagellated amastigote form, which thrives and multiply within the phagolysosomes through a complex parasite–host interaction (Mauricio and Jonatan, 2015). Current control strategies for VL rely on anti-leishmanial drugs such as pentavalent antimonials, amphotericin B (AmB), miltefosine, paromomycin, etc., but they are far from satisfactory because of their cost, toxicity as well as unpleasant side effects, longer dose schedule with variable efficacies (Lodge *et al*., 2006). The situation has further worsened with the emergence of resistance against current anti-leishmanial drugs in various regions of endemicity (Kedzierski, 2010). Hence, in the present situation, there is an urgent need to develop an effective vaccine against VL. Although vaccination against VL has received limited attention as compared to cutaneous leishmaniasis (CL), till date, there is no commercial vaccine against any human parasitic disease including leishmaniasis (Croft *et al*., 2006). Leishmania parasite follows a digenetic life cycle it results in significant antigenic diversity, which

****Corresponding author: Dr. Sabiha Imran,***
Department of Biotechnology, Faculty of Engineering and Technology, ManavRachna International University, Faridabad, Haryana, INDIA

ultimately hampered the passage of vaccine development against VL, therefore, the knowledge of such antigenic diversity is of utmost importance (Kumari, 2008). Several approaches utilized for identification of potential antigens, which can be targeted as suitable vaccine candidate (Figure 1).

Strategies for Vaccine Development against Visceral Leishmaniasis	
1	Classical Approach
	Whole Parasite Vaccine
	Native protein Based vaccine
2	Molecular Approach
	DNA Vaccine
	Polyprotein Vaccine
	Recombinant Vaccine
	Liposomised Vaccine
	Salivary Antigen based vaccine
3	Alternative Approach
	Mutant Vaccine
	Synthetic Vaccine

Figure 1. Approaches to develop vaccines against visceral leishmniasis

Among them, proteomics attract the most since it addresses several unanswered questions related to microbial pathogens, including its development, evolution, and pathogenicity. Recent advancement in computational biology further simplifies our knowledge regarding the in-depth study of parasite. T-cell epitope prediction via bioinformatics analysis of protein sequences has been proposed as another alternative for rationale vaccine development (Lundegaard, 2007). The concept that CD8+ T lymphocytes could be important in protection and long-lasting resistance to infection has opened up a new strategy in Leishmania vaccine design known as "polytope vaccine" (Palatnik de Sousa, 2008). Its major advantages include greater potency, can be controlled better, can be designed to break tolerance, can overcome safety concerns associated with entire organisms or proteins, etc. *Leishmania* species employ distinct mechanisms to elude effector arms of the immune system, including inhibition of NO- and ROS-mediated macrophage killing and phagolysosomal fusion, inhibition of cell-mediated immunity via blocking of antigen presentation and cytokine production, and recruitment of regulatory T cells or other interleukin-10 (IL-10)-producing cells (Nylen *et al*., 2007).

Various approaches to develop vaccines against Visceral Leishmaniasis

Live/killed whole parasite vaccine

As the researchers started culturing promastigote form of parasite in artificial media, the concept of live vaccination came into existence. A number of large-scale vaccination trials were conducted during the 1970s and 1980s in Israel, Iran, and the Soviet Union with a higher success rate. However, standardization and quality control are the major issues associated with live vaccines because parasites used for vaccination losses its infectivity due to repeated sub-culturing. Therefore, the focus of vaccine development program was shifted toward killed organisms in the early 90s (Handman, 2001). Use of whole killed parasites with or without adjuvant was proposed for both therapeutic as well as for prophylactic purposes (Basyoni, 2012). The vaccination trial conducted in a Brazilian population showed excellent protection with up-regulation of IFN-γ and absence of IL-4, an indicator of long-lasting Th1-type immune response (Castellano *et al*., 2009).

Protein fractions based vaccine

Selection of suitable vaccine candidates seems to be a difficult task due to the multitude of antigens that has been evaluated with varied success rate depending on their formulation and the type of animal model used (Sinha, 2011). Complete protection has not been achieved so far due to the complexity of the parasite, which generates poly-specific response (Campos, 2001).

Recombinant protein vaccine

With the advancement in recombinant DNA technology, several leishmanial molecules, either species or life cycle stage specific, were extensively studied as a promising vaccine candidate in the form of recombinant proteins. The major advantages associated with these proteins are in terms of purity as well as yield.Recombinant proteins, either alone or combined with adjuvant or with bacteria/recombinant virus as a delivery vehicles (Maroof, 2012). Have also been tested as vaccines in preclinical studies. There have been significant efforts in recent time to identify recombinant antigens that can protect against *Leishmania* infection in experimental models. Some of these antigens include kinetoplastid membrane protein-11, (Agallou, 2011) sterol 24-c-methyltranferase (Goto, 2007) amastigote specific protein A2, (Ghosh, 2001) cysteine proteinase B, (Rafati, 2006). *Leishmania*-activated C kinase, promastigote surface antigen 2, (Benhnini, 2009) nucleoside hydrolase (Al-Wabel, 2007) and surface expressed glycoprotein gp63 (Connell, 1993). Although most of these recombinant antigens have been tested in animal models for their immunogenicity and protective efficacy, only a few have progressed to clinical trials in non-human primates, dogs or in preclinical human studies

Polyprotein vaccine

Due to the genetic polymorphism in the mammalian immune system, a multicomponent vaccine thought to elicit a better protective immune response (Goto, 2011). Therefore, multicomponent or polyprotein preparations such as Q protein, Leish-111f, Leish-110f, KSAC, etc., came into existence that had been demonstrated to afford better protection against experimental VL. Among these, Q protein containing five genetically fused antigenic determinants from Lip2a, Lip2b, H2A and P0 proteins, was initially assessed along with either BCG or CpG-ODN in mice and dogs (Molano, 2003), against L. donovani challenge (Parody, 2004).

DNA vaccines

Besides proteins, DNA had also been extensively utilized as a means of vaccine delivery, which reformed the area of vaccinology. Here, genes encoding the target proteins are cloned into a mammalian expression vector, which is injected either intradermally or intramuscularly leading to induction of Th1 responses, resulting in strong cytotoxic T-cell immunity. Safety, stability, long-term protection, ease of administration, and cost effectiveness are the major issues associated with this form of vaccine delivery. Several molecules were evaluated using this approach such as A2, PapLe22, P36LACK, ORFF, KMP-11 proteophosphoglycan (PPG), etc., in different animal models with significant level of protection. A2(Ghosh, 2001) and ORFF (Sukumaran, 2003) when administered as a DNA vaccine were found to be significantly protective in BALB/c

mice against VL, which induced both humoral and cellular immune responses. Zanin *et al*. (Zanin, 2007). immunized mice with a NH/A2 DNA vaccine resulted in increased IFN-γ, IL-4, and IL-10 levels associated with edema and increased parasite loads. Das *et al*. (Das, 2014) very recently have developed a DNA vaccine using conserved proteins from various Leishmania species and found to be immunogenic inducing CD4+ and CD8+ T-cell responses in genetically diverse human populations of different endemic regions.

Heterologous prime boost vaccine

Different researchers utilized another strategy known as heterologous DNA-prime protein-boost (HPB) approach for some VL vaccine antigens such as cysteine proteinases, GP63, etc., which have also shown success but are yet to reach the level of clinical trials.Rafati *et al*. (Rafati *et al*., 2005 and 2006) observed that vaccination mainly elicited antigen-specific IgG2a antibodies, suggesting the induction of a Th1 immune response. Liposomised delivery of parasite protein Liposome formulations have been adopted as a drug delivery system against Leishmania infection so as to induce an elevated immune response owing to their adjuvant property (Bhowmick S, 2010) thus can offer a new approach to the development of VL vaccines wherein it may induce a sustained Th1 immune response. This approach using L. donovani promastigote membrane antigens (LAg) encapsulated in positively charged liposomes were found to induce significant protection against experimental VL by Afrin *et al*. (Afrin, 2002). In another study, using liposomised recombinant membranous protein – GP63 of L. donovani, there was a long-term protection against VL in BALB/c mice (Bhowmick, 2008)

Sandfly's salivary antigen as vaccine

Salivary proteins of vector-sandfly also fetch attraction as suitable anti-VL vaccine candidates. They received little attention in spite of the fact that salivary proteins from the vector are also delivered to the host during natural transmission of the pathogen and sometimes found immunomodulatory for the host (Basyoni, 2012). Several salivary proteins of Phlebotomus spp. and Lutzomyia spp. such as PpSP15, maxadilan, LJM17, LJM19, and LJM143 have been reported as potent immunogens inducing lymphocytic infiltration with up-regulation of IFN-γ and IL-12 (Gomes, 2008 and Collin, 2009). Similarly, immunization with other two salivary proteins – LJL143 and LJM17 generated strong Th1 responses in dogs with distinct cellular infiltration of CD3 + lymphocytes and macrophages (Collin, 2009). Therefore, these proteins may further be explored in conjunction with potent parasite proteins for vaccination studies. Despite these different approaches offer a variable degree of efficacy, several problems still hampers its feasibility due to variations in immunogenicity and due to genetic variation in host as well as in pathogen (Kumar R and Engwerda, 2014). Therefore, despite of numerous recombinant proteins that have been suggested as potential vaccine candidates, to date barely few have reached to clinical trials (Duthie, Reed, 2014).

Newer Alternative Strategies for Developing Anti-Leishmanial Vaccine

Live mutant vaccine: Attenuation of virulent Leishmania parasites through defined genetic alteration is a new area in vaccine research since the perception of vaccination suggests that the more similar a vaccine is to the natural disease, better is the generation of protective immune response (Silvestre, 2008). Poor long-term immunity is the major issue with various recombinant vaccines tested so far while whole cell killed vaccines showed variable efficacy. Consequently, live-attenuated vaccine attracts the immunologists, since, it offers a complete milieu of antigens to the antigen presenting cells (APCs), therefore, providing an optimal polarization of CD4+ T-cells, resulting in better immune response (Sporri, 2005). Also, they assure persistence of antigen that may allow the generation of antigen-specific effector and memory cells, which react immediately following infection (Foulds, 2006). Centrin, a growth regulated gene was deleted from the amastigote stage of the L. donovani parasite and was subjected to evaluation of its prophylactic potential (Silvestre, 2008). The $LdCen^{-/-}$ parasite was found to be safe and protective in mice and hamsters against virulent challenge(Selvapandiyan A, 2009) and is under exploration for further development as potential vaccine against VL. Very recently, Dey *et al*. (Dey, 2013) have demonstrated another knock out – Ldp27 (−/−) parasites to be safe and can provide protective immunity against both homologous and heterologous challenge with stimulation of both Th1-type CD4+ and CD8+ T-cells. Since, effector T-cell population requires continuous stimulation for excellent protection; it can be well accomplished through live-attenuated vaccines.

Synthetic peptide vaccine

Recent developments in blending of bioinformatics with vaccinology has revolutionized and expedited this area. Sequencing of large number of pathogen genome and increase in nucleotide and protein sequence databases accelerate the pace of vaccine development program. Although, killed or attenuated parasites are utilized for most of the existing vaccines, protective immune response is more often triggered by small amino acid sequence (peptides). More recent bioinformatic approaches utilizes number of algorithms for predicting epitopes, HLA-binding, transporter of antigen processing (TAP) affinity, proteasomal cleavage, etc., in order to explore the use of peptide epitopes with the highest probability of inducing protective immune responses. Synthetic peptide vaccines offer several advantages over other vaccine types like absence of any potentially infectious material, ability to include multiple epitopes, minimization of the amount and complexity of an antigen, economical scale up and decreased chance of stimulating a response against self-antigens.

Conclusion

The main scientific issues in the design of a Leishmania vaccine are no different from those for any other vaccine. They include specificity, the class or type of response induced, and the induction of long-term immunological memory. On a more positive note, there is currently rapid progress in our understanding of the molecular nature of potential vaccine candidates and of the mechanisms that determine disease-preventing immune responses. Several strategies such as genomic databases, evolutionary relationships, three-dimensional structure of proteins, presence of specific protein domains, primary structure of proteins, etc., have been applied to knowhow novel interacting partners in order to validate the presumed interactions.Notwithstanding, there is a feeling of

renewed optimism in the scientific community that a Leishmania vaccine is achievable.

REFERENCES

Afrin, F., Rajesh, R., Anam, K., Gopinath, M., Pal, S., Ali, N.. 2002. Characterization of Leishmaniadonovani antigens encapsulated in liposomes that induce protective immunity in BALB/c mice. *Infect Immun;* 70:6697–70610

Agallou, M., Margaroni, M., Karagouni, E. 2011. Cellular vaccination with bone marrow-derived dendritic cells pulsed with a peptide of *Leishmaniainfantum* KMP-11 and CpG oligonucleotides induces protection in a murine model of visceral leishmaniasis. *Vaccine* ; 29: 5053–5064

Al-Wabel, M.A, Tonui WK, Cui L, Martin SK, Titus RG.2007. Protection of susceptible BALB/c mice from challenge with *Leishmania major* by nucleoside hydrolase, a soluble exo-antigen of *Leishmania*. *Am J Trop Med Hyg* ; 77: 1060–1065.

Basyoni MM.2012.*Leishmania* vaccines updates. *PUJ* ; 5(1):1–10

Benhnini F, Chenik M, Laouini D, Louzir H, Cazenave PA, Dellagi K.2009. Comparative evaluation of two vaccine candidates against experimental leishmaniasis due to *Leishmania major* infection in four inbred mouse strains. *Clin Vaccine Immunol* ; 16: 1529–1537.

Bhowmick S, Mazumdar T, Sinha R, Ali N.2010.Comparison of liposome based antigen delivery systems for protection against Leishmaniadonovani. *J Control Release* ;141:199

Bhowmick S, Ravindran R, Ali N.2008.GP63 in stable cationic liposomes confers sustained vaccine immunity to susceptible BALB/c mice infected with Leishmaniadonovani. *Infect Immun*;76:1003–1510

Campos-N A, Porrozzi R, Greeson K, Coler RN, Webb JR, Seiky YA, *et al*.2001.Protection against cutaneous leishmaniasis induced by recombinant antigens in murine and nonhuman primate models of the human disease. *Infect Immun* ;69:4103–810

Castellano LR, Filho DC, Argiro L, Dessein H, Prata A, *et al*. 2009. Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-gamma production. *Hum Immunol*;70: 383–390.

Chappuis F, Sundar S, Hailu A, Ghalib H, Rijal S, Peeling RW, *et al*. 2007.Visceral leishmaniasis: what are the needs for diagnosis, treatment and control? *Nat Rev Microbiol*; 5:873–8210.

Collin N, Gomes R, Teixeira C, Cheng L, Laughinghouse A, Ward JM, *et al*. 2009.Sand fly salivary proteins induce strong cellular immunity in a natural reservoir of visceral leishmaniasis with adverse consequences for *Leishmania*. *PLoSPathog*; 5(5):e1000441

Connell ND, Medina-Acosta E, McMaster WR, Bloom BR, Russell DG.2009. Effective immunization against cutaneous leishmaniasis with recombinant bacilleCalmette-Guerin expressing the Leishmania surface proteinase gp63. *Proc Natl AcadSciUSA*; 90: 11473–11477.

Croft SL, Sundar S, Fairlamb AH.2006. Drug resistance in leishmaniasis. *ClinMicrobiol Rev*;19:111–2610

Das S, Freier A, Boussoffara T, Das S, Oswald D, Losch FO, *et al*. 2014.Modular multiantigen T cell epitope-enriched DNA vaccine against human leishmaniasis. *SciTranslMed* ;6:234ra56

Dey R, Dagur PK, Selvapandiyan A, McCoy JP, Salotra P, Duncan R, *et al*. 2013.Live attenuated Leishmaniadonovani p27 gene knockout parasites are nonpathogenic and elicit long-term protective immunity in BALB/c mice. *J Immunol* ;190(5):2138–491

Duthie MS, Reed SG.2014.The emergence of defined subunit vaccines for the prevention of leishmaniasis. *Curr Trop Med Rep*;1(3):154–6210

Foulds KE, Wu CY, Seder RA.2006. Th1 memory: implications for vaccine development. *Immunol Rev* 211:58–6610

Ghosh A, Zhang WW, Matlashewski G. 2001.Immunization with A2 protein results in a mixed Th1/Th2 and a humoral response which protects mice against Leishmaniadonovani infections. *Vaccine*20:59–6610

Gomes R, Teixeira C, Teixeira MJ, Oliveira F, Menezes MJ, Silva C, *et al*. 2008.Immunity to a salivary protein of a sandfly vector protects against the fatal outcome of visceral leishmaniasis in a hamster model. *Proc Natl AcadSci U S*A ;105:7845–5010

Goto Y, Bhatia A, Raman VS, Liang H, Mohamath R, Picone AF, *et al*.2011.KSAC, the first defined polyprotein vaccine candidate for visceral leishmaniasis.*Clin Vaccine Immunol*;18:1118–2410

Goto Y, Bogatzki LY, Bertholet S, Coler RN, Reed SG. 2007.Protective immunization against visceral leishmaniasis using Leishmania sterol 24-c-methyltransferase formulated in adjuvant. *Vaccine* ; 25: 7450–7458

Handman E, Symons FM, Baldwin TM, Curtis JM, Scheerlinck JP.1995. Protective vaccination with promastigote surface antigen 2 from *Leishmania major* is mediated by a TH1 type of immune response. *Infect Immun ;* 63*:* 4261–4267.

Handman E.2001.Leishmaniasis: current status of vaccine development. ClinMicrobiolRev ; 14:229–4310

Hasker E, Singh SP, Malaviya P, Picado A, Gidwani K, Singh RP, *et al*. 2012.Visceral leishmaniasis in rural Bihar, India. *Emerg Infect Dis*;18(10):1662–410.

Kedzierski L.2010.Leishmaniasis vaccine: where are we today? *J Glob Infect Dis*; 2(2):177–8510

Kumar R, Engwerda C.2014.Vaccines to prevent leishmaniasis. *Clin Trans Immunol*;3:e13.10

Kumari S, Kumar A, Samant M, Sundar S, Singh N, Dube A.2008. Proteomic approaches for discovery of new targets for vaccine and therapeutics against visceral leishmaniasis. *Proteomics ClinAppl* ; 3:372–8610

Lodge R, Diallo TO, Descoteaux A.2006.*Leishmaniadonovani*lipophosphoglycan blocks NADPH oxidase assembly at the phagosome membrane. Cell Microbiol; 8:1922–3110

Lukes J, Mauricio IL, Schonian G, Dujardin JC, Soteriadou K, Dedet JP, *et al*.2007. Evolutionary and geographical history of the *Leishmaniadonovani* complex with a revision of current taxonomy. *Proc Natl AcadSci U S A*; 104:9375–8010

Lundegaard C, Lund O, Kesmir C, Brunak S, Nielsen M. 2007.Modeling the adaptive immune system: predictions and simulations. Bioinformatics ;23(24):3265–7510

Maroof A, Brown N, Smith B, Hodgkinson MR, Maxwell A, Losch FO *et al*.2012.Therapeutic vaccination with recombinant adenovirus reduces splenic parasite burden in experimental visceral leishmaniasis. *J Infect Dis*; 205: 853–863

Mauricio M R and Jonatan E. 2015. Neglected Tropical Diseaes, Bioinformatics and Vaccine, *J Infect Dis*., 211(2):175-177

Molano I, Garcıa Alonso M, Miron C.2015. A *Leishmaniainfantum* multi-component antigenic protein mixed with live BCG confers protection to dogs experimentally infected with *L. infantum*.*Vet ImmunolImmunopathol*; 2003 92:1–1310

Negar S,Tahereh T and SimaR .2016.Post-Genomics and Vaccine Improvement for *Leishmania, FrontMirobiol*.; 7: 467.

Nylen S., *et al*. 2007. Splenic accumulation of IL-10 mRNA in T cells distinct from $CD4^+$ $CD25^+$ (Foxp3) regulatory T cells in human visceral leishmaniasis. *J. Exp. Med*; 204: 805– 817

Palatnik de Sousa CB. 2008.Vaccines for leishmaniasis in the fore coming 25 years.Vaccine ; 26:1709–2410

Parody N, Soto M, Requena JM, Lonso CA.2004. Adjuvant guided polarization of the immune humoral response against a protective multicomponent antigenic protein (Q) from *Leishmaniainfantum*. A CpG +Q mix protects Balb/c mice from infection. *Parasite Immunol* ; 26:283–9310

Rafati S, Nakhaee A, Taheri T, Taslimi Y, Darabi H, Eravani D, *et al*. 2005.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*; 23(28):3716–2510

Rafati S, Zahedifard F, Nazgouee F. 2006.Prime-boost vaccination using cysteine proteinases type I and II of *Leishmaniainfantum* confers protective immunity in murine visceral leishmaniasis. *Vaccine*; 24: 2169–2175.

Selvapandiyan A, Dey R, Nylen R, Duncan R, Sacks D, Nakasi HL. 2009. Intracellular replication-deficient Leishmaniadonovani induces long lasting protective immunity against visceral leishmaniasis. *J Immunol*; 183:1813–2010

Silvestre R, Cordeiro-da-Silva A, Ouaissi A.2008. Live attenuated *Leishmania* vaccines: a potential strategic alternative. *Arch ImmunolTherExp (Warsz)* ;56(2):123–610

Sinha S., Sundaram S., Singh A. P., Tripathi A. 2011. A gp63 based vaccine candidate against visceral leishmaniasis. *Bioinformation*; 5, 320–325

Sporri R, Reis E, Sousa C. 2005.Inflammatory mediators are insufficient for full dendritic cell activation and promote expansion of CD4+ T cell populations lacking helper function. *Nat Immunol* ; 6:163–7010

Sukumaran B, Tewary P, Saxena S, Madhubala R.2003.Vaccination with DNA encoding ORFF antigen confers protective immunity in mice infected with Leishmaniadonovani. *Vaccine,* 21:1292–910

Zanin FH, Coelho EA, Tavares CA, Marques-da-Silva EA, Costa MM, Rezende SA, *et al*. 2007. Evaluation of immune responses and protection induced by A2 and nucleoside hydrolase (NH) DNA vaccines against Leishmaniachagasi and Leishmaniaamazonensis experimental infections. *Microbes Infect*; 9:1070–710

****