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Altercating Leishmaniasis: Cultivating Improved Vaccines

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Abstract The exponentially rising cases of Leishmaniasis over the past decades, has attracted scientists and clinicians to mitigate the contagious infection by modern treatments and new generation vaccinations. *Leishmania*, the causative pathogen of this infection predominantly expresses its diseased condition in form of cutaneous leishmaniasis. Although systemic leishmaniasis, is a more deadly form, but cases of its manifestation are limited; thereby staging the focus onto more intricate study of cutaneous leishmaniasis. Designing a vaccine against Leishmania has witnessed more than two generations of vaccine, but each attempt has failed more than it's cases of success. The proposed drawbacks root to the difficulty in ensuring an efficient transition of research from simple laboratory experiments carried out *in-vitro*/*in-vivo* in small animals to that of the humans. There have been extensive case studies of this disease and thus every immunological aspect has been our focus. This paper is an attempt to decipher the ideas and methodologies adopted till date and further target the genetic spans and molecular moieties, for crafting an alternative vaccine based on efforts to mock the pathogenic pathways.

Keywords: *cutaneous, proteomics, recombinant DNA, macrophage, life cycle, live-attenuated vaccines*

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1. Introduction

Leishmaniasiscaused by the genus ***Leishmania***, has been classified as one of the most neglected diseases [5]. For many years, the public health influence of leishmaniasis has been underestimated [6]. The expansion of leishmaniasis and the sharp rise in occurrence is related to environmental changes and migration of non-immune people to endemic areas [6]. *Leishmania* parasites are responsible for a family of diseases, collectively known as leishmaniasis, with discrete clinical features ranging from cutaneous lesions to fatal systemic diseases [5,6]. There are several **diverse forms of leishmaniasis** in individuals (Figure 1). Some people have a silent infection of leishmania, without any signs or symptoms of the infection.

Figure 1. Formsof Leishmaniasis: Ranging from acute to chronic disease manifestation.

The visceral form, also known as black sickness or **kala-azar** is an Indian name, meaning "black fever" (darkening of the skin)in Asia, is characterized by prolonged fever, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, pancytopenia, and hypergammaglobulinemia and is complicated by serious infections [3]. After recovery, some patients also develop **post-kala-azar dermal leishmaniasis** [PKDL], which requires prolonged and expensive treatment. Natural transmission of leishmania is carried out by a certain species of sand fly of the genus *Lutzomyia*[15].

An increasing resistance to the currently used drugs, their side effects and more importantly, the lack of efficient vaccines has been the major boost for growing research in this field. [19]. Precisely, intensive study of the parasite has also demanded athorough analysis of the host-pathogen interactions for designing the ultimate **HERO ANTI-LEISHMANIA** vaccine. Although, studies of several model organisms especially mice, heralded the scope of solid protection that can be reached upon by immunization with defined subunit vaccines [either protein or DNA] or heat-killed parasites, but, to date the test results of such vaccines have seen unsatisfactory fates. [10].

1.1. Leishmanial Stages

Leishmania spp., known to be a digenetic parasites, involves two hosts, a vertebrate-is the **amastigote (**the

intracellular form in the vertebrate host) and an invertebrate- the **promastigote (**the extracellular form in the sand-fly) [5]. Amastigotes can be isolated from the blood of an infected host when the female sand fly bites, and in the sand fly gut they are designated as promastigotes during the time of their residence. In the gut they multiply by binary fission; followed byanterior movement into the proboscis, and are introduced into the vertebrate host upon the bite of sand fly to its host. The injected promastigotes inside the host are phagocytized while feeding and develop into intracellular amastigotes [5,14].

Amastigotes are set up in cells of the reticulo-endothelial system of vertebrate hosts [skin, bone marrow, liver, spleen, and lymph nodes]. In large mononuclear cells, instances of their presence in the bloodstream have been confirmed [4,29,32].

After initial ingestion of blood meal from the host, promastigotes development in the sand fly takes 8-20 days, and followed by multiplication in the midgut-hindgut, they move forward to the pharynx where they produce a partial or complete blockage of the sucking apparatus.When such a sand fly tries to feed it,an upward movement of bolus of metacyclic promastigotes occurs which is then inserted into the bite wound [10,21,26].

1.2. Alternative Pathogenicity of Parasites

L. majorlacks the gene for the potential target enzymes,and in such a case pathogenicity is rendered by the presence of Serine protease inhibitors [ISPs]. The ISPs have inhibitory effects against vertebrate macrophage serine proteases, such as neutrophil elastases [39]. Case of ISPs interfering in trypsin-like action of sand fly midguts has been reported [25]. Moreover, a few parasites of *Leishmania* family also have mechanisms that intensify their resistance to proteolytic attack without inhibiting the overall proteolytic activity in the midgut [28].

The results of **Secundino et al** [47] focused on L. major surface PPG (proteophosphoglycan) as the likely key molecule conferring resistance of fully developed procyclic promastigotes to the activity of digestive enzymes; showing that a parasite line lacking surface PGs is more susceptible to killing than the wild type when exposed to blood-fed midgut lysates [12]. However, addition of purified PPG is known to classically reverse this effect [22]. Further the sand fly midgut proteolytic enzymes are negative effectors on the *Leishmania* growth in the vector and therefore, can be categorized as attractive targets for vector-based transmission blocking strategy [32].

1.3. Proteins and Peptides Involved

Innate immune response-Defensins, cationic antibacterial peptides have been defined in the fat body and the midgut. [70] Transcripts coding for numerous other putative components of the innate immune response have been discovered in the sand fly midgut, such as pattern recognition proteins, a glycin-rich protein and serpins. Antioxidant enzyme's homologues, which can regulate midgut epithelial immunity and dictate the fate of bacterial and parasitic infections in mosquitoes, have also been found [21,23,24,43,46,47]. Recently it has been established that in *L. longipalpis*, depletion of Caspar (a putative negative regulator of immune deficiency signaling pathway) by RNAi prior to blood feeding, results in significant reduction of populations of both *L. mexicana* and *L. infantum*. The major vivid inference is that, the growth and spread of leishmaniasis is crosschecked by several levels of systemic immune response [35,42,47].

1.4. Alteration of Macrophage Signaling Molecules by Leishmania

Two major strategies adopted by *Leishmania* to alter the macrophage signaling pathway are- inhibition of proteins that play a positive role in immune cell activation and activation of molecules known to play key roles in thenegative regulation of immune cell signaling and function (Figure 2) [14].

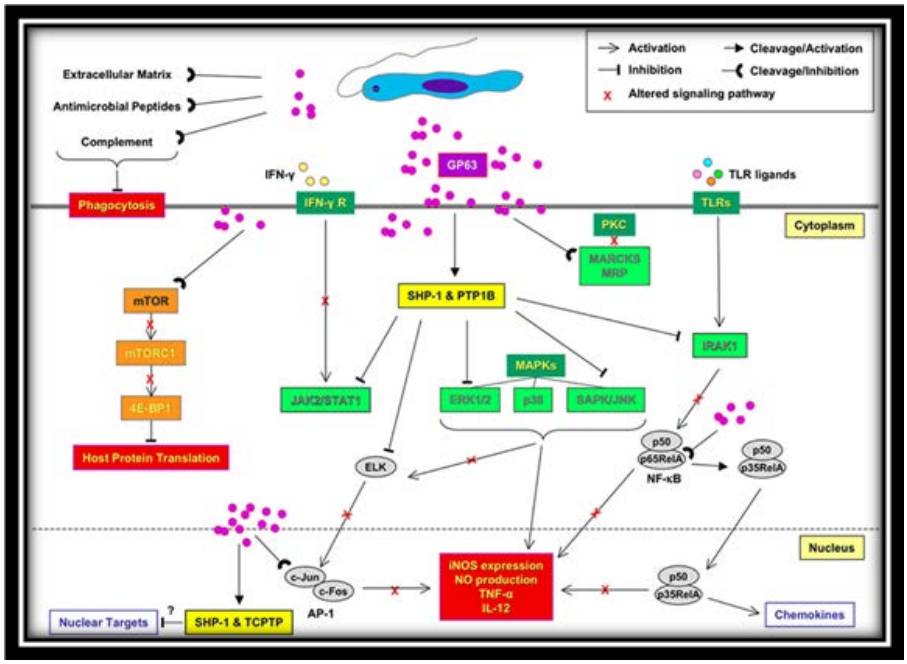

Figure 2. Signaing Pathways activated post *Leishmania* infection [Sourced from :www.frontiresin.org]

Critical Survival Strategies developed by parasite to ensure smooth residence inside the macrophages are:

- The parasite is able to induce the chemokine expression and production by host immune cells [36]. It has been shown that the infection of mice with *L. major* upregulated the gene expression of several chemokines [RANTES/CCL5, MIP-1α/CCL3, IP-10/CXCL10, and MCP-1/CCL2] in cells collected from the footpad and their draining lymph nodes [16].
- *L. major* infection results in higher levels of several chemokines [RANTES, MIP-1α, MIP-1β/CCL4, IP-10, MCP-1, and MIP-2/CXCL1] in cells charecterised by their fast recruitement at the site of infection [61]. To add imporance to this finding it has been shown that in general a large population of chemokines are monocyte chemoattractants, recruiting macrophages to infected tissues and immobilizing the parasite [26].
- Protein kinase C [PKC] signaling acts as a key regulatior of macrophage functioning; e.g. cytokines such as IFN-γ and TNF-α , both crucial in driving several functions including NO production and oxidative burst [8,10]. Promastigote LPG has been identified as an antagonist of PKC [11] which is manifested by the ceompetitive binding of LPG to the regulatory domain of PKC which contains the Diacyl glycerol(DAG), Ca^{+2}, and phospholipid binding sites [29].
- *Leishmania* encounters macrophages which are capable of producing deadly free radicals such as NO [64] and reactive oxygen intermediates(ROIs) [35]. To escape this, the parasite has the ability to block the JAK/STAT signaling pathway in response to IFN-γ stimulation, supressing the induction of NO [41]. However, cases have been reported where *Leishmania* affects JAK2 phosphorylation by rapidly activating host SHP-1 leading to the subsequent inhibition of IFN-λ-induced JAK2 phosphorylation [30]. It has been observed that IFN-γ unresponsiveness to stimulation can be due to the inhibition of the IFN-γ receptor [IFN-γR] complex formation [21,27,33].
- As a key by-passing policy *Leishmania* parasite develop tactics to deactivate several MAPK members post parasite intrusion into macrophages or by activating stimuli that follow infection [24]. For instance, the phagocytosis of *L. donovani* promastigotes by naive macrophages does not lead to the activation of any of the three MAPKs [Erk1/2, JNK, p38.
- In respect to p38, stimulation with a CD40 antibody (mimicing the macrophages-T cell interaction) has been effective in generating nonresponsiveness toMAPK, even when macrophages are infected with *L. major*, [31]. Leishmanicidal functionsgets impaired in context ofp38 inactivation with supression iNOS2 expression and NO production [31].
- In order to inhibit gene expression of proinflammatory cytokines and microbicidal molecules, *Leishmania* develop several strategies to interfere with transciption factors that bind to the promoters of these genes [31,32].

1.5. Modulation of Toll-Like Receptor Signaling by *Leishmania*

TLR family members are classical mediators of both innate and adaptive immune response through their potential ability of recognition of pathogen-associated molecular patterns [PAMPs] [17].

- GPI-anchored proteins, are a group of parasite-derived molecules involved in TLR binding and activation. TLR signalling in primary exposureinduces tolerance to secondary TLR stimulation [19].This was later known to be mediated by the ability of GPI-mucin to induce the expression and activation of the serine/threonine phosphatase PP2A that acts on cellular IRAK-1, MAPKs, and IκB causing their inhibition and leading to tolerance [11]. The induction of PP2A was shown to require p38 and NF-κB, the very same molecules PP2A is induced to inhibit, therefore giving rise to an auto regulatory loop [10].
- LPG of *Leishmania* is another GPI-anchored protein detected by TLRs. It has been shown that LPG of *L. major* directly binds to TLR2 of Macrophages and NK cells [11,12] and that LPG of *L. donovani* is also detected by TLR2 of activated Macrophages [13].

Also the **non-GPI-related ligands** are sensed by the TLRs. DNA of *T. cruzi*, *T. brucei* and *Babesiabovis*, are able to activate macrophages and Dendritic cells [18,19] in lieu of their unmethylated CpG motifs [20] which can be detected by TLR9 [21,22].

TLRs as potent parasitic PAMPs detector along with multi-faceted responsiveness in cases of successful infections raise questions associated with silent entry to target cells, suggesting parasitic techniques evading of host TLR signaling pathways.

It is clear that TLRs play a crucial role in mounting innate and adaptive immunity against invading pathogens [1,13]. Nevertheless, the effects of blocking TLR-TLR-ligand interactions on the ability of the immune system to fight off other pathogens that can be/become present and thereby needs to be taken into serious consideration.

2. Implications of Leishmanial-diagnosis

2.1. Antigen Detection

De Colmenares et al. [20] from Spain have reported two polypeptide fractions of 72-75 kDa and 123 kDa in the urine of kala-azar patients. The sensitivities of the 72-75-kDa fractions were 96%, and the specificities were 100%.

A new latex agglutination test [KATEX] for detecting leishmanial antigen in urine of patients with VL has showed sensitivities between 68 and 100% and a specificity of 100% in preliminary trials. The antigen is detected quite early during the infection and the results of animal experiments suggest that the amount of detectable antigen tends to decline rapidly following chemotherapy [17,24].

2.2. Antibody Detection

Conventional methods for antibody detection include gel diffusion, complement fixation test, indirect

hemagglutination test, IFA test, and counter current immunoelectrophoresis [2,13]. However, aside from practical difficulties at peripheral laboratories, the sensitivities and specificities of most of the above tests have been the limiting factors [36]. In 1988, a modified DAT was reported to be useful in kala-azar and is being used in several countries of endemicity [27].

2.3. Western Blot

Specific antibodies can also be detected by Western blotting [38]. In this diagnostic method, promastigotes of *L. donovani* are grown to log phase and lysed and the soluble protein is used as sample [18]. With Western blotting, one can find even minor antigenic differences among various organisms and thus detect cross-reactive antigens [28]. Though, the process is time consuming, technically cumbersome, and expensive [8] tonsils, and skin; and even as widely disseminated disease [4, 8]. The diagnostic principles remain essentially the same as those for non-HIV-infected patients [8]. For HIV patients, the sensitivity of antibody-based immunologic tests like the IFA test and ELISA is low [3,4]. Since the parasite load is quite heavy in these patients, the presence of leishmania amastigotes in the bone marrow can be verified.

3. Overview of Vaccine Development

The comparatively straightforward leishmanial life cycle and the point that recovery from a primary infection provides the host resistance to subsequent infections, indicate the feasibility of vaccination [10].

- First generation vaccines; represented by whole killed parasites, have been proposed as both prophylactic and therapeutic vaccines [32]. These whole-cell killed vaccines with undefined and variable in potency, have been fated with limited success rates [11].
- Second generation vaccines consist of recombinant proteins, poly-proteins, DNA vaccines or dendritic cells loaded with peptides derived from leishmanial antigens [33]. A GPI-anchored membrane protein gp46 or Parasite Surface Antigen 2(present in most *Leishmania* species) has also been identified as suitable vaccine candidate, [34]. PSA-2 results in macrophage invasion through the interaction of its leucine rich repeats with complement receptor [35]. Immunization with the native polypeptides derived from promastigotes protected mice against infection, [36] in contrast to vaccination with a recombinant protein derived from either promastigotes or amastigotes protein showed lack of protective efficacy [37]. DNA vaccination conferred protection in mice when used as either prophylactic[38] or therapeutic vaccines [39]. For instance, one more extensively tested antigen is the *Leishmania* homologue for receptors of activated C kinase [LACK] that is expressed throughout leishmanial life cycle [40]. Immunization with LACK appears to promote the expansion of IL-4 secreting T cells twisting the response towards detrimental Th2 responses [41].
- Statistically, only one second generation vaccine, Leish-111f (a single polyprotein composed of three molecules fused; the *L. major* homologue of eukaryotic thiol-specific antioxidant [TSA], the *L. major* stress-inducible protein-1 [LmSTI1] and the *L. braziliensis* elongation and initiation factor [LeIF] [25]), has been assessed in clinical trials [15,45]. Initial immunisation trials in mice confirmed that Leish-111f was able to protect mice against *L. major* and *L. amazonensis* infection[36,46].

Sand fly saliva contains an array of molecules able to interfere with the host immune responses, [60] therefore; immunity against saliva components may indirectly enhance anti-leishmanial immunity [36]. Immunization with molecules present in saliva, such as maxadilan [22] or a 15-kDa protein, SP15 [36] also induced protection against cutaneous leishmaniasis [22]. Recently, it has been shown that vector salivary proteins, in particular LJM19, protect hamster from VL, [44] and immunization of dogs with salivary antigens led to the development of high IgG2 antibody levels and significant IFN-γ production [35].

3.1. Live-attenuated Leishmania-An alternative vaccine approach

The live-attenuated vaccine exhibits a complete spectrum of antigens to the antigen presenting cells, ultimately leading to a better immune response in comparison to that of a subunit vaccine.

Avirulent microorganisms crafted by a defined genetic manipulation, restricting the reversion of pathogenicity. Only a minority of attenuated strains has been tested so far with many outcomes, and the live attenuated, antileishmanial vaccine is still at its initial stages of expansion. Recently, *L. donovani* centrin (a calcium-binding cytoskeletal protein involved in the duplication of centrosomes in higher eukaryotes) null mutants [LdCEN$^{-/-}$] have been reported to have selective growth arrest in the amastigote stage of development, but were viable in culture as promastigotes [22]. These mutants were unable to survive *in vitro* in human macrophages and animals vaccinated with LdCEN$^{-/-}$ mutants were protected against homologous as well as heterologous challenge [23]. It has lately been confirmed that *L. major* phosphomannomutase [PMM] deficient mutants were able to defend susceptible mice beside infection via an increased magnitude of T cell responses and suppression of IL-10 and IL-13 production early during infection [44].

Another example of an attenuated vaccine that displayed protective efficacy is *L. infantum SIR2* single knockout strain, [77] however; the integral problem of this strain is the existence of the second *SIR2* allele making reversion to virulence a possible occurrence.

3.2. Impediments for Vaccine Designing

Vaccination is the best answer to question pertaining to infectious diseases. Leishmaniasis should be manageable by immunization, seems irrefutable in context of the existing experimental evidences. Therefore, understanding the shortcomings of the marketed vaccines is crucial to future stages of anti-leishmanial vaccine designing.

Insights into antileishmanial immunity explain the catastrophic existence of the first generation vaccines. *Peters et al* demonstrated that sand fly transmission of parasites abodisrupts vaccine-induced protective immunity

[32].Mice vaccinated with killed parasites, were stubborn to a needle challenge, and were then prone to the sand fly inoculum inferring that the responses in vaccinated mice required for defense were either not generated or not preserved. On the other hand, mice that had cured the primary lesion were protected against sand fly challenge, and the swiftness of the response proposes that the protective response was not resultant from the central memory, rather from an effector pool of T cells that could have been preserved by the persistent parasites. This data provides a foundation for the inclusion of sand fly saliva components, which are specific to natural infection. It has been established that inoculation of killed parasites into immune mice leads to a loss of infection induced immunity [38].

Selection of vaccine candidates has continued to be a really difficult problem. An excess of antigens have been calculated with mixed success dependent on the formulation and the animal model used for testing it. However, complete protection has not been reached so far and immunization has generally led only to partial protection. In addition, the opinions on the nature of the vaccine have been at odds. Some claim that a vaccine against leishmaniasis should be molecularly defined, while others say for a live attenuated vaccine.

One of the major problems a vaccine faces against leishmaniasis, are alterations in virulence factors among the species as well as in the immune responses that they induce. For example, LPG is a virulence factor for *L. major*, [45] but not for *L. mexicana* [46] During the *L. major* infection the defensive role of Th1 responses has been established, but *L. amazonensis* is able to survive in the presence of Th1 responses, and causes minimal disease in the complete absence of T cells [47].Studies explaining parasite-vector interactions have become the basis for novel approaches to reduce transmission of numerous insect-borne ailments.

4. Conclusion

The main concern over the issue of development of vaccines for leishmania is no different from other diseases that are encountered. They target the specificity, the class or type of response made, and the promotion of long-term immunological memory of the disease. This review gives an insight into the developing world of vaccinations where first line of vaccination and subsequently the second line of vaccination could not bring us the possible results as desired. This review focuses on advances in anti-leishmania vaccine development over the current years and examines current problems that are hampering vaccine development and implementation in leishmaniasis.

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