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Review Article

Prospects of Vaccine in Leishmaniasis

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Abstract

The clinical manifestations of leishmaniasis depend on the interaction between the genetics of the parasite and the genetics of the host. However, in human infections, the host population is heterogeneous and the parasites are not clonal, and this makes it difficult to dissect out the relative contributions of the parasite and the host. The current data indicate that susceptibility to leishmaniasis is controlled by many genes, including TNF (tumor necrosis factor), MHC (major histocompatibility complex), NRAMP1 (natural resistance associated macrophage protein 1) and others of unknown function. Susceptibility, resistance, and disease patterns probably depend on complex interactions between these genes. However, despite the wealth of information regarding the genetics of the parasite and the experimental immunology of the disease, there is currently no licensed vaccine against Leishmania and control measures rely on chemotherapy to alleviate disease and on vector control to reduce transmission. A major vaccine development program aimed initially at cutaneous leishmaniasis is under way. Studies in animal models and humans have been evaluating the potential of genetically modified live attenuated vaccines, as well as a variety of recombinant antigens or the DNA encoding them. The availability, in the near future, of the DNA sequences of the entire human and Leishmania genomes will extend the vaccine program by mapping the host susceptibility genes to allow the vaccine to be targeted to the population most in need of protection. This review has focused on the factors elucidated for host and parasite governing disease outcome and also several of the Leishmania vaccination strategies employed to date.

Key words: Leishmania, Leishmaniasis, Host-susceptibility genes, Vaccines

Introduction

Leishmaniasis continues to have a major impact on much of the world's population. Worldwide, there are 2 million new cases each year and 1/10 of the world's population is at risk of infection[1]. The disease is endemic throughout parts of Africa, India, the Middle East, southern Europe, and Central and South America, and epidemics are also well recognized. With the emergence of the human immunodeficiency virus (HIV) epidemic, leishmaniasis has surged as a reactivating infection in AIDS patients in many parts of the world[2]. There are six species of *Leishmania* recognized to cause disease in humans which are very similar morphologically but produce strikingly different pathological responses such as visceral leishmaniasis, oriental sore or espundia. In the vertebrate host, *Leishmania* parasites survive and multiply intracellularly in mononuclear phagocytes as nonmotile amastigotes. It is often assumed that the type of disease is determined by the species of the parasite, but this may be an oversimplification. The genetics and immunocompetence of the host may be equally important for some parasite species[3]. Infection does not necessarily lead to disease, and a significant proportion of the population in areas of endemic infection may harbor subclinical infections, which may only declare itself upon immunosuppression such as in AIDS patients[3,4]. Now it is obvious that the clinical manifestations or outcome of infection caused by *Leishmania* are determined by interactions between the host and parasite, which are governed by their genomes. It is therefore very exciting to note that both host and parasite genomes have been targeted for sequence analysis. The genetic information from both

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human and parasite and the emergence of new tools like microarray techniques will allow us to gain an in-depth understanding of the interaction between parasite virulence factors and host response factors. Until now, the factors determining susceptibility or resistance to visceral leishmaniasis remain partly unclear, but it is reasonably assumed that the genetics of the host may play a major role in the outcome of infection. The genes on the part of host so far causally related to leishmaniasis are genes encoding Tumor necrosis factor alpha and beta, Major histocompatibility complex and Natural resistance associated macrophage protein[5].

Investigation of this parasite's interaction with the host's immune system may lead to a better understanding of how *Leishmania* infection can be resisted or the evasion strategies of these parasites which influence susceptibility of the host. Improved understanding of these areas should assist in the development of an effective prophylactic vaccine, capable of inducing the specific immune responses required to battle this parasite[6].

Despite substantial efforts spent, there are no licensed vaccines available against leishmaniasis to date and control measures rely on chemotherapy to alleviate disease and on vector control to reduce transmission. In order to develop an effective vaccine, it is important to understand the mechanisms of the immune response to *Leishmania* infection so that the vaccine can be engineered to induce a protective response rather than one that could result in susceptibility to the parasite. A major vaccine development program aimed initially at cutaneous leishmaniasis is under way[7]. Leishmanization with living parasite was an early attempt but studies in animal models and humans are evaluating the potential of genetically modified live attenuated vaccines, as well as a variety of recombinant antigens or the DNA encoding them[8]. The vaccine program also focuses on new adjuvants, including cytokines and delivery systems to target the T helper type 1 immune responses required for the elimination of this intracellular organism[9,10]. The availability, in the near future, of the DNA sequences of the human and *Leishmania* genomes will extend the vaccine program further[11]. It is assumed that new vaccine candidates such as parasite virulence factors will be identified and host susceptibility genes will be mapped to allow the vaccine to be targeted to the population most in need of protection[12,13].

Host Parasite Relationship

Leishmaniasis constitutes a diverse collection of human diseases ranging from spontaneously healing ulcer, sometimes disfiguring cutaneous or mucocutaneous lesions to visceral disease affecting the haemopoetic organs. Like many other infectious diseases specially those ones which are chronic in nature, the clinical manifestations of leishmaniasis depend on the interaction between the genetics of the parasite and the genetics of the host[3]. But unfortunately in human leishmaniasis, the host population is heterogeneous and the parasites are not clonal, and this makes it difficult to dissect out the relative contributions of the parasite and the host. However, the outcome of infection is largely dependent on the ability of the host to mount a protective T-helper-1 (Th_1) response versus the ability of the parasite to evade and manipulate the host's immune system[14]. Macrophages and effector molecules, dendritic cells (DC), T-helper cells (CD4+ T cells), cytotoxic T cells (CD8+ T cells), natural killer (NK) cells and cytokines are all, in one way or another, considered to play important roles in the immune response to *Leishmania* infection[15].

Much of current knowledge of the contribution of host genetics to the pathogenesis of leishmaniasis comes from mouse models using cloned parasite lines and inbred mice, but these systems have many limitations and serve only as guides for exploration the facts in humans. During its development in the sandfly gut as a motile promastigote, a biochemical modification of the parasite's glycolipid coat occurs. This very transformation protects the parasite from rapid lysis via the mammalian complement system. Further, in an elegant example of molecular mimicry, the parasite uses the host complement receptor to gain access into the hostile environment of the phagolysosome and thrives. Depending on the species of the parasite, resistance to infection is generally associated with a T-helper-1 immune response that activates macrophages to kill intracellular *Leishmania* in a nitric oxide-dependent manner. Conversely, disease progression is generally associated with a T-helper-2 response that activates humoral immunity. It is expected that with the advent of the complete sequence of the human genome, it would facilitate the mapping of human genes controlling susceptibility to leishmaniasis. On the other hand, the elucidation of the complete sequence of the *Leishmania* genome, which is well under way, should facilitate the discovery of genes which determine parasite virulence[11, 16] .

In the search of susceptibility genes, several host genes have been identified using genetic approaches in both mice and humans. The early discovery that susceptibility to *L. donovani, Salmonella enterica* serovar Typhimurium, and *Mycobacterium bovis* was partly controlled by a single gene on mouse chromosome 1 led to the isolation from humans and mice of the gene encoding natural resistance-associated macrophage protein 1 (*NRAMP1*)[3,17]. Disappointingly, recent data tend to indicate that this gene may not play a role in human leishmaniasis, in contrast to the wealth of prosperous data obtained in mice. The precise biological function of NRAMP1 is not yet known, but a closely related protein, NRAMP2, is a transporter of iron from endosomes to the cytoplasm[18]. While no association has been seen in humans between allelic forms of NRAMP1 and leishmaniasis, an understanding of its action may still help explaining the

peculiar ability the parasite has of surviving the harsh environment of the phagosome.

The molecular pathogenesis of mucocutaneous leishmaniasis, which might cause grossly disfiguring complication of a previously healed cutaneous infection, still remains a puzzle. Again host genetic factors have now been implicated in the pathogenesis of mucosal disease. A study in a Venezuelan population demonstrated that particular alleles encoding the cytokines "tumor necrosis factor" (TNF alpha and TNF beta) were associated with significantly increased relative risks (3.5 and 7.5 respectively) of mucocutaneous disease[19]. TNF alpha was over expressed from the allele associated with disease, an observation that fits well with other reports of high concentration of TNF alpha found in patients with leishmaniasis. This cytokine releasing gene was further implicated by a mouse study where one of the two receptors of TNF alpha was ablated by means of gene targeting. The mice lacking the receptor could not heal cutaneous ulcers despite being able to control parasite replication[20]. It has leaded the speculation that obviously, molecules other than the TNF receptors must be involved in susceptibility to disease.

For a disease in which healing and recovery depend on the induction of cell-mediated immunity, it is not surprising that the major histocompatibility complex (MHC) has been implicated intimately in its susceptibility. In mouse model, it was shown that different MHC haplotypes were associated with different degrees of susceptibility to visceral leishmaniasis. A role for the MHC in human cutaneous leishmaniasis has also been described in and is supported by a genetic linkage study in mice[21,22]. These data add to the wealth of evidence supporting a role of the MHC in resistance to a variety of infectious diseases including leprosy, schistosomiasis, malaria, hepatitis B infection, and the progression of HIV infection to AIDS[23,24].
The mouse model of infection with *Leishmania major* helped to explore the cellular basis of this phenomenon. Heinzel et al. correlated that the outcome of infection is determined by the nature and magnitude of T cells and cytokine response early in infection. In the infected inbred mice, production of IFN gamma by Th1 and natural killer (NK) cells mediate resistance, while, expansion of IL-4 producing Th2 cells confers susceptibility[25]. Environment produced by these different cytokines released by two subsets of T helper cells in turn recruits and activates different immune effector cells[26]. Moreover, once established, these responses become mutually exclusive to a large extent. In this logical way the type of T helper cell response determines the susceptibility or resistance to *Leishmania* infection.

In summary, the current data indicate that susceptibility to leishmaniasis is controlled by many genes, including TNF, the MHC, NRAMP1, and others of unknown function. Susceptibility, resistance, and disease patterns probably depend on complex interactions between these genes[5].

Vaccines in leishmaniasis
Historically, cutaneous leishmaniasis has been the focus of vaccination attempts, probably because it has been known since antiquity that individuals who had recovered from infection with *Leishmania* confers immunity to reinfection suggests that control of leishmaniasis by vaccination may be possible. Further, the observation that unlike some other parasites, *Leishmania* can be grown in cell-free media with ease and the use of killed parasites as skin-test antigens (leishmanin) for diagnosis in humans during the past several decades have prompted scientists to try using the killed parasites, with or without adjuvant, as vaccines or for immunotherapy. In addition, different recombinant molecules, either parasite fractions or genetically engineered organisms (i.e. *Leishmania* made avirulent by removing specific genes, or bacteria carrying and expressing *Leishmania* genes), are being investigated as potential future vaccines against leishmaniasis. The 'first-generation' vaccines, composed of killed parasites with or without adjuvant, have been derived using an empirical approach. The 'second-generation' vaccines have been genetically constructed, using a more rational approach. However, there are no vaccines available at present to control any form of leishmaniasis, despite considerable efforts. Studies of the immunopathogenesis and mechanisms of protective immunity, mainly derived from animal models of experimental *Leishmania*sis, have defined a number of features that should be met by an effective vaccine. To date, there is no vaccine against *Leishmania* in routine use anywhere in the world. Several vaccine preparations are in more or less advanced stages of testing.

Live Non-attenuated Vaccines: Nicolle and Manceau (1908) established the culture conditions able to support the growth of promastigotes[27] and since then live organisms have been used for making controlled infections if not for vaccination in true sense[28]. 'Leishmanization', the deliberate inoculation of virulent *Leishmania* from the exudate of a cutaneous lesion, is the oldest vaccination against cutaneous leishmaniasis and has been practiced for centuries. This is because it has long been well established that recovery from cutaneous leishmaniasis is followed by long lasting immunity to the disease. Large-scale vaccination trials in the form of making controlled infection using live promastigotes were carried out in the Soviet Union and Israel with a high percentage of successful lesion development[29]. The success of this strategy relied critically on the viability and infectivity of the injected organisms. Organisms which had lost virulence were shown to induce delayed-type hypersensitivity but did not protect vaccinated persons from subsequent natural infections[30,32]. Further, the use of live vaccines for *Leishmania*sis has had many problems, including the development of large uncontrolled skin lesions, exacerbation

of psoriasis and other skin diseases, and even immunosuppression as determined by low responses to the diphtheria, pertussis, and tetanus triple vaccine[7,32]. Consequently, the use of live virulent organisms for vaccination was discontinued, and in the 1990s the focus shifted to killed organisms.

Killed vaccines: Killed *Leishmania* is an appealing vaccine candidate in terms of its stable biochemical composition and antigenicity, low cost and safety[33]. There have been several studies on killed *Leishmania*, with or without adjuvants, as a vaccine. Extensive vaccination trials with a cocktail of five killed *Leishmania* stocks or a single strain of *L. amazonensis* in Brazil and Ecuador have demonstrated significant protection from natural infection[34,35]. 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 Th1 type, which may indicate a potential to protect from natural infection[36].

In Iran, a mixed BCG-*L. major* killed vaccine has also undergone clinical trials for safety and efficacy. In one study there was little difference in disease incidence between the group vaccinated with BCG alone and the group given BCG and vaccine. A second study showed that in the longer term, the vaccine combination provided better efficacy than BCG alone, suggesting that BCG may have had only a transient immunostimulatory effect[35].

Live-Attenuated Vaccines: The relative merits of live-attenuated vaccines versus killed vaccines have been a constant subject of debate in relation to many antimicrobial and viral vaccines. As discussed above for *Leishmania*, the most notable arguments have been those concerned with immunogenicity, efficacy, safety, ease of production and distribution, and stability. Genetic modification of *Leishmania* parasites to reduce virulence, yet maintain immunogenicity, is of current interest in *Leishmania* vaccine research. It is an appealing approach as attenuated parasites also called 'knock-out parasites' closely mimic natural infection that may lead to similar immune responses without the danger associated with infection with live virulent parasites. Due to advances in molecular biology and the genomic sequencing of *L. major*, the attenuation of *Leishmania* parasites by removing, blocking or replacing essential genes is a possibility[37,38]. It is now possible to generate parasites lacking genes essential for long-term survival in the mammalian host, such as the gene encoding the enzyme dihydrofolate reductase-thymidylate synthetase (DHFR-TS) [39]. These organisms can invade and undergo a limited number of replications in macrophages without producing disease.

The use of attenuated organisms 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 delivered by the transient infection, the immune responses may be skewed even more toward a Th1 protective response than in natural infection. Although attenuated vaccines offer a novel approach to immunization against leishmaniasis however, there are fears that the parasite may revert back to a virulent form and also the issues of logistics for their large-scale production and distribution in the field are concern.

Recombinant and Synthetic Vaccines: The newer vaccines under consideration comprise recombinant DNA-derived antigens and peptides. Some of the target antigens are species and life cycle stage specific, while others are shared by promastigotes and amastigotes. Some are conserved among *Leishmania* species, while others are not. Since T cells recognize peptides derived from cytosolic proteins bound in the MHC class I groove or peptides derived from the lysosomal compartment bound in the MHC class II groove on the antigen-presenting cell surface, it would appear that virtually any parasite protein might function as an antigen, regardless of its location in the parasite.

Recombinant antigens can be delivered 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[40]. DNA vaccines present a multitude of advantages over other vaccine strategies and several features have made them an appealing alternative. Not only are they fast, simple and cheap to produce on a large scale, they are also temperature stable making storage and transport easier and cheaper as there is no need for a cold-chain of distribution[41.]

Naked DNA Vaccines: Immunization with naked DNA is a new approach, which promises to revolutionize the prevention and treatment of infectious diseases[42,43]. The gene encoding the vaccine candidate is cloned in a mammalian expression vector, and the DNA is injected directly into muscle or skin[44]. Surprisingly, the plasmid DNA is taken up by cells and translocated to the nucleus, where it is transcribed into RNA and then translated in the cytoplasm. The efficiency of uptake and the expression of plasmid DNA must be extremely low, but there is abundant evidence that it is sufficient to provoke immune responses in both T and B cells[45].

Non-protein Antigens: Early studies on vaccine development indicated that glycolipids such as the *Leishmania* lipophosphoglycan (LPG) provided excellent protection[46]. Protection depended on the use of adjuvants such as liposomes or *Corynebacterium parvum* and on the integrity of

the molecule. Immunity was known to be T-cell mediated, but T cells were not thought to recognize or present nonprotein antigens. Today, it is accepted that many novel and interesting microbial antigens including mycobacterial glycolipids can be recognized by T cells and that these antigens are presented to T cells by a special subset of MHC class I proteins known as CD1[47]. In this context, it may be rewarding to reevaluate the potential of LPG as a vaccine candidate.

Issues to be addressed for successful vaccine preparation

Protective antigens which induce the appropriate immune responses leading to host protection must be properly identified. The advent of gene cloning and monoclonal antibodies opened up the possibility of identifying and characterizing relevant protein antigens and most importantly producing them in unlimited amounts. With the continuous research, it is now clear that many antigens may combine to elicit protective immune responses.

Safety related to vaccine is another very vital issue. One of the worries in vaccine development is the possibility that a particular vaccine may exacerbate the disease associated with infection or cause pathological reactions due to cross-reactivity between host and parasite antigens. Another nightmare is the possibility of unleashing the disease due to incomplete attenuation or inactivation of the organisms. There is also the possibility that attenuated organisms may revert and cause disease in immunocompromised individuals.

Delivery and Adjuvants related to vaccine administration is very essential for its efficacy. The ability of certain bacteria and other particles to be taken up by the dendritic cells, coupled with the ability to express foreign genes in bacteria has made them attractive delivery vehicles for vaccines. Such a vaccine could exploit attenuated bacteria such as *Salmonella* or BCG, which are already in use as vaccines with demonstrated safety and immunogenicity. BCG has been used successfully for anti-*Leishmania* immunotherapy in South American patients without side effects. BCG vectors carrying gp63 have also been used successfully to induce protection in the *L. major* system.

On the contrary, although the number and type of adjuvants have expanded, their mechanism of action has remained largely mysterious and empirical. Adjuvants are believed to contribute to the amplitude of the immune response and to its quality off course. The importance lies in a judicious choice of adjuvant for *Leishmania* vaccines that require Th1 responses for protection.

Several particulate adjuvants or delivery systems have been tested for use with *Leishmania* vaccines including liposomes, microparticles, immunostimulating complexes, and micelles formed by intrinsically adjuvanted lipopeptides. One of the most promising adjuvants involves the use of some of "nature's adjuvants" i.e., soluble cytokines which are known to promote Th1 immune responses. Among these, IL-12 is essential for the induction and maintenance of Th1 immune responses by *Leishmania* vaccines, including DNA vaccines[48]. An interesting approach to the targeting of the immune response has been the coupling of the potent antigen-presenting ability of dendritic cells to the delivery of proinflammatory cytokines to the local site of response. For this purpose, adoptive transfer of dendritic cells engineered by retroviral infection to secrete IL-12 has been used to augment the effect of vaccination with dendritic cells pulsed with *L. donovani* antigen.

Conclusion

Leishmaniasis is a major cause of morbidity and mortality worldwide. Although there has been tremendous achievement in formulation of new chemotherapeutic agents to combat this infection but preparation of appropriate vaccines for leishmaniasis is still under trial. Unlike chemotherapy, vaccination is usually a "one-shot" affair. This makes it cheaper, and the easier logistics of administration lead to much better compliance. Vaccines have the advantage that they can be administered both in the prophylactic and therapeutic modes. The main scientific issues in the design of a *Leishmania* vaccine are no different from those for any other vaccine. They include specificity, the type of response induced, and the induction of long-term immunological memory. Like many other vaccines, the "rules of the game" for achieving these goals are still far from clear. The optimistic note is that, there is currently rapid progress in our understanding of the molecular nature of potential vaccine candidates and of the mechanisms of immune responses that determine disease prevention. However, manipulation of these responses in a reliable way is still at an early stage. Notwithstanding these caveats, there is a feeling of renewed optimism in the scientific community that a *Leishmania* vaccine is achievable.

References

1. World Health Organization. 1998. Advances in the battle against *Leishmania*sis. TDR News. 57**:**2
2. World Health Organization. 1991. AIDS, *Leishmania*sis dangers of clash highlighted. TDR News. 36**:**1-11.
3. Blackwell, J. M. 1996a. Genetic susceptibility to *Leishmania*l infections: studies in mice and man. Parasitology. 112**:**S67-S74.
4. Badaro, R., E. M. Carvalho, H. Rocha, A. C. Queiroz, and T. C. Jones. 1986. *Leishmania donovani*: an opportunistic microbe associated with progressive disease in three immunocompromised patients. Lancet. 1**:**647-649.
5. Handman, E. 2001. *Leishmania*sis: Current Status of Vaccine Development. Clinical Microbiology Reviews. 14(2):229-243.
6. Zambrano-Villa, S., Borjas, D**.,** Carrero J., et al. 2002. How protozoan parasites evade the immune response.

Trends Parasitol. 18(6):272–278.

7. Modabber, F. 1995. Vaccines against *Leishmania*sis. Ann. Trop. Med. Parasitol., 89**:**83-88.

8. Dunning, N. 2009. *Leishmania* vaccines: from leishmanization to the era of DNA technology. Bioscience Horizons. 2**:**73-82.

9. Schijns, V. E. J. C. 2000. Immunological concepts of vaccine adjuvant activity. Curr. Opin. Immunol., 12**:**456-463.

10. Rivier, D., P. Bovay, R. Shah, S. Didisheim, and J. Mauel. 1999. Vaccination against *Leishmania major* in a CBA mouse model of infection: role of adjuvants and mechanism of protection. Parasite Immunol., 21**:**461-473.

11. Blackwell, J. M. 1997. Parasite genome analysis. Progress in the *Leishmania* genome project. Trans. R. Soc. Trop. Med. Hyg., 91**:**107-110.

12. Greenblatt, C. L. 1980. The present and future of vaccination for cutaneous *Leishmania*sis. Prog. Clin. Biol. Res., 47**:**259-285.

13. Alarcon, J. B., G. W. Waine, and D. P. McManus**.** 1999. DNA vaccines: technology and application as anti-parasite and anti-microbial agents. Adv. Parasitol., 42**:**344-410.

14. Liese J, Schleichera U, Bogdan C. 2008. The innate immune response against Leishmania parasites. Immunobiology. 213**:**377–387.

15. Vanloubbeeck Y, Jones D. 2004. The immunology of Leishmania infection and the implications for vaccine development. Ann N Y Acad Sci., 1026**:**267–272.

16. Ivens, A. C., and D. F. Smith. 1997. Parasite genome analysis. A global map of the *Leishmania major* genome: prelude to genomic sequencing. Trans. R. Soc. Trop. Med. Hyg., 91**:**111-115.

17. Blackwell, J. M. 1996b. Structure and function of the natural-resistance-associated macrophage protein (Nramp1), a candidate protein for infectious and autoimmune disease susceptibility. Mol. Med. Today., 2**:**205-211.

18. Fleming, M. D., C. C. Trenor III, M. A. Su, D. Foernzler, D. R. Beier, W. F. Dietrich, and N. C. Andrews. 1997. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat. Genet., 16:383-386.

19. Lara, M. L., Z. Layrisse, J. V. Scorza, E. Garcia, Z. Stoikow, J. Granados, and W. Bias. 1991. Immunogenetics of human American cutaneous *Leishmania*sis. Study of HLA haplotypes in 24 families from Venezuela. Hum. Immunol., 30**:**129-135.

20. Nashleanas, M., S. Kanaly, and P. Scott. 1998. Control of *Leishmania major* infection in mice lacking TNF receptors. J. Immunol., 160**:**5506-5513.

21. Leclercq, V., M. Lebastard, Y. Belkaid, J. Louis, and G. Milon. 1996. The outcome of the parasitic process initiated by *Leishmania infantum* in laboratory mice: a tissue-dependent pattern controlled by the *Lsh* and MHC loci. J. Immunol., 157:4537-4545.

22. Wassom, D. L., and E. A. Kelly. 1990. The role of the major histocompatibility complex in resistance to parasite infections. Crit. Rev. Immunol., 10**:**31-52.

23. Mann, D. L., R. P. Garner, D. E. Dayhoff, K. Cao, M. A. Fernandez-Vina, C. Davis, N. Aronson, N. Ruiz, D. L. Birx, and N. L. Michael. 1998. Major histocompatibility complex genotype is associated with disease progression and virus load levels in a cohort of human immunodeficiency virus type 1-infected Caucasians and African Americans. J. Infect. Dis., 178**:**1799-1802.

24. Reed, S.G., and P. Scott. 1993. T-cell and cytokines responses in *Leishmania*sis. Curr. Opin. Immunol., 5**:**524-531.

25. Abbas, A. K., Murphy, K. M., and A. Sher. 1996. Functional diversity of helper T lymphocytes. Nature. 383**:**787-793.

26. Roberts, L. J., E. Handman, and S. J. Foote. 2000. *Leishmania*sis in the new genetic era. Br. Med. J., 321**:**801-804.

27. Nicolle, C. H. 1908. Cultures des corps de Leishman isoles de la rate dans trois cas d'anemic sptenique infantile. Bull. Soc. Pathol. Exot., 1**:**121.

28. Kellina, O. I. 1981. Problems and current lines in investigations on the epidemiology of *Leishmania*sis and its control in the USSR. Bull. Soc. Pathol. Exot., 74**:**306-318.

29. Kellina, O. I. 1965. Changes in virulence of strains of *Leishmania tropica major*. Med. Parasitol., 6**:**701-708.

30. Serebryakov, V. A., S. K. H. Karakhodznaeva, and M. D. Dzhumaev. 1972. Effect of *Leishmania*l vaccinations on the dynamics of immunity to diphteria in conditions of secondary revaccination with adsorbed pertussis-diphteria-tetanus vaccine. Med. Parasit. Mosk., 41**:**303-309.

31. Giunchetti, R., et al. 2008. Antigenicity of a whole parasite vaccine as promising candidate against canine leishmaniasis. Res Vet Sci., 1**:**106–112.

32. Marzochi, K. B., M. A. Marzochi, A. F. Silva, N. Grativol, R. Duarte, E. M. Confort, and F. Modabber. 1998. Phase 1 study of an inactivated vaccine against American tegumentary *Leishmania*sis in normal volunteers in Brazil. Mem. Inst. Oswaldo. Cruz, 93**:**205-212.

33. Armijos, R. X., M. M. Weigel, H. Aviles, R. Maldonado, and J. Racines. 1998. Field trial of a vaccine against New World cutaneous *Leishmania*sis in an at-risk child population: safety, immunogenicity, and efficacy during the first 12 months of follow-up. J. Infect. Dis., 177:1352-1357.

34. Mendonca, S. C. F., P. M. de Luca, W. Mayrink, T. G. Restom, F. Conceicao-Silva, A. M. Da-Cruz, A. L. Bertho, C. A. Da Costa, O. Genaro, V. P. C. P. Toledo, and S. G. Coutinho. 1995. Characterization of human T lymphocyte-mediated immune responses induced by a vaccine against American tegumentary *Leishmania*sis. Am. J. Trop. Med. Hyg., 53**:**195-201.

35. Momeni, A. Z., T. Jalayer, M. Emamjomeh, A. Khamesipour, F. Zicker, R. L. Ghassemi, Y. Dowlati, I. Sharifi, M. Aminjavaheri, A. Shafiei, M. H. Alimohammadian, R. Hashemi-Fesharki, K. Nasseri, T. Godal, P. G. Smith, and F. Modabber. 1999. A randomised, double-blind, controlled trial of a killed *L. major* vaccine plus BCG against zoonotic cutaneous *Leishmania*sis in Iran. Vaccine. 17**:**466-472.

36. Roberts M. 2006. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. Br Med Bull., 75–76**:**115–130.

37. Titus, R. G., F. J. Gueiros-Filho, L. A. de Freitas, and S. M. Beverley. 1995. Development of a safe live *Leishmania* vaccine line by gene replacement. Proc. Natl. Acad. Sci., USA. 92**:**10267-10271.

38. Matzinger, P. 1998. An innate sense of danger. Semin. Immunol., 10**:**399-415.

39. Palatnik-de-Sousa C. 2008. Vaccines for leishmaniasis in the fore coming 25 years. Vaccine. 26**:**1709–1724.

40. Seder, R. A., and S. Gurunathan. 1999. DNA vaccines designer vaccines for the 21st century. N. Engl. J. Med., 341**:**277-278.

41. Gurunathan, S., C. Wu, B. L. Freidag, and R. A. Seder. 2000. DNA vaccines: a key for inducing long-term cellular immunity. Curr. Opin. Immunol., 12**:**442-447.

42. Donnelly, J. J., J. B. Ulmer, J. W. Shiver, and M. A. Liu. 1997. DNA vaccines. Annu. Rev. Immunol., 15**:**617-648.

43. Hasan, U. A., A. M. Abai, D. R. Harper, B. W. Wren, and W. J. Morrow. 1999. Nucleic acid immunization: concepts and techniques associated with third generation vaccines. J. Immunol. Methods. 229**:**1-22.

44. McConville, M. J., A. Bacic, G. F. Mitchell, and E. Handman. 1987. Lipophosphoglycan of *Leishmania major* that vaccinates against cutaneous *Leishmania*sis contains an alkylglycerophosphoinositol lipid anchor. Proc. Natl. Acad. Sci., USA. 84**:**8941-8945.

45. Moody, D. B., T. Ulrichs, W. Muhlecker, D. C. Young, S. S. Gurcha, E. Grant, J. P. Rosat, M. B. Brenner, C. E. Costello, G. S. Besra, and S. A. Porcelli. 2000. CD1c-mediated T-cell recognition of isoprenoid glycolipids in *Mycobacterium tuberculosis* infection. Nature. 404**:**884-888.

46. Hoskins, T. W., J. R. Davies, A. J. Smith, C. L. Miller, and A. Allchin. 1979. Assessment of inactivated influenza-A vaccine after three outbreaks of influenza A at Christ's Hospital. Lancet. i: 33-35.

47. Aebischer, T., M. Wolfram, S. I. Patzer, T. Ilg, M. Wiese, and P. Overath. 2000. Subunit vaccination of mice against new world cutaneous *Leishmania*sis: comparison of three proteins expressed in amastigotes and six adjuvants. Infect. Immun., 68**:**1328-1336.

48. Scott, P., and G. Trinchieri. 1997. IL-12 as an adjuvant for cell-mediated immunity. Semin. Immunol., 9**:**285-291.