The utility of rhesus monkey (*Macaca mulatta*) and other non-human primate models for preclinical testing of *Leishmania* candidate vaccines - A Review

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Leishmaniasis causes significant morbidity and mortality, constituting an important global health problem for which there are few effective drugs. Given the urgent need to identify a safe and effective Leishmania *vaccine to help prevent the two million new cases of human leishmaniasis worldwide each year, all reasonable efforts to achieve this goal should be made. This includes the use of animal models that are as close to leishmanial infection in humans as is practical and feasible. Old world monkey species (macaques, baboons, mandrills etc.) have the closest evolutionary relatedness to humans among the approachable animal models. The Asian rhesus macaques (*Macaca mulatta*) are quite susceptible to leishmanial infection, develop a human-like disease, exhibit antibodies to* Leishmania *and parasite-specific T-cell mediated immune responses both in vivo and in vitro, and can be protected effectively by vaccination. Results from macaque vaccine studies could also prove useful in guiding the design of human vaccine trials. This review summarizes our current knowledge on this topic and proposes potential approaches that may result in the more effective use of the macaque model to maximize its potential to help the development of an effective vaccine for human leishmaniasis.*

Key words: non-human primates - experimental leishmaniasis - *Leishmania* vaccine development

Leishmaniasis is one of the major infectious diseases primarily affecting some of the poorest regions of the world. The disease is endemic in 88 countries, and the World Health Organization estimates that it is a threat to 350 million people with a worldwide prevalence of 12 million cases. Among the annual incidence of 2 million new cases of human infections, 0.5 million are life-threatening visceral leishmaniasis (VL) (www.who.int/tdr/diseases). Cutaneous leishmaniasis (CL) caused by highly pathogenic parasites is also characterized by its chronicity, latency and tendency to metastasize, resulting in recurrent skin lesions with the potential for mucosal involvement. It should be noted that an estimated 2.4 million disability adjust life years, in addition to 59,000 lives, were lost to leishmaniasis in 2001 alone (Davies et al. 2003). Concerns about chemotherapy failure for both VL and CL are exacerbated by geographical variation in antimonial treatment regimens, severity of disease and sensitivity of *Leishmania* species. In addition, no proven successful vaccine for controlling human leishmaniasis is in routine use (Davies et al. 2003, Kedzierski et al. 2006). Moreover, at least 20 genetically heterogeneous *Leishmania* species infect humans and each of them has a unique epidemiological pattern, such that two or more parasite species are often sympatric in sylvan areas of the Neotropics (Grimaldi & Tesh 1993). These data explain the limited success of current control strategies based on conventional measures (such as vector reduction and elimination of infected reservoir) for American leishmaniasis.

The solid protective immunity observed in humans following convalescence to CL formed the basis for practice of active immunization, beginning with deliberate inoculation of virulent organisms ("leishmanization") in centuries past and continuing with vaccination using a crude antigen preparation obtained from inactivated ("killed") promastigotes of one or various species of *Leishmania*, formulated either with or without BCG (bacillus of Calmette and Guerin) as an adjuvant (Grimaldi 1995). While accumulated experience with mass vaccination both in the ex-USSR and in Israel has clearly shown that a virulent strain of *Leishmania* must be used for vaccination to succeed (Gunders 1987), several Phase III trials testing the potential efficacy of various crude vaccine approaches have given conflicting results. Overall, the results vary from 0-75% efficacy against CL and little (< 6%) or no protection against VL (Grimaldi 1995, Coler & Reed 2005). Although host genetics can have dramatic effects on T-cell responses to existing vaccines (Lambert et al. 2005), several technical problems (including inadequate information about the quality, stability and potency of the antigens) may provide explanation for some of the variation in efficacy observed in those human vaccine studies. Nevertheless, most experts believe that a preventive vaccine will be essential if this disease is ever to be controlled worldwide (Coler & Reed 2005, Tabbara 2006, Kedzierski et al. 2006, Palatnik-de-Souza 2008, Silvestre et al. 2008).

The current effort to develop improved vaccines for leishmaniasis has led to the need for appropriate animal models in which to test candidate vaccines (Hein & Griebel 2003). There are reminders that the results from rodent models do not automatically translate to humans

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(MacGregor et al. 1998). The use of non-human primates (NHP) as animal models for the study of human diseases (including immunological studies and drug and vaccine-development studies against infectious diseases) has become increasingly important (Campos-Neto et al. 2001, Delgado et al. 2005, Giavedoni 2005, Gibbs et al. 2007, Nikolich-Žugich 2007, Souza-Lemos et al. 2008). For instance, the SIV-macaque model is widely used for testing vaccine and therapeutic strategies prior to conducting human clinical trials (Nathansson et al. 1999, Hu 2005). This review aims to provide insight into the current knowledge on vaccine studies against leishmaniasis, with emphasis on studies involving vaccination and experimental infection in monkeys.

Vaccine studies against leishmaniasis

A major international research effort over the past 20 years has resulted in the identification of various *Leishmania* antigen candidates for second and third-generation vaccines (Coler & Reed 2005, Palatnik-de-Souza 2008). Information about a multitude of immunization approaches representing all of the major vaccine design strategies, including vaccines using live genetically attenuated parasites, subunit proteins/peptides in adjuvants, naked DNA and infectious vectored vaccines expressing genes coding for specific leishmanial antigens and combinations thereof has been given in recent review articles (Coler & Reed 2005, Tabbara 2006, Kedzierski et al. 2006, Palatnik-de-Souza 2008, Silvestre et al. 2008). Many of these vaccines have been tested for immunogenicity and protective efficacy in a variety of experimental models (such as inbred laboratory rodents, dogs and NHP). Depending on the particular vaccine approach and animal model used, varying degrees of protective immunity have been achieved, as determined by the level of parasite burden in infected sites and/or lesion size following infectious challenge.

Vaccination strategies are based on the immunology of *Leishmania* infection (Vanloubbeeck & Jones 2004, Von Stebut 2007). On the basis of compelling evidence that both $CD4^+$ (including multifunctional Th1 cells and central memory $CD4^+$ T-cells) and $CD8^+$ T-cells are key players in the immune response to leishmaniasis (Reed & Scott 2000, Zaph et al. 2004, Darrah et al. 2007), the scientific community has focused considerable efforts on the development of prophylactic vaccines that elicit T-cell responses (Rhee et al. 2002, Tapia et al. 2003, Sharma et al. 2006, Dondji et al. 2008) with the premise that such interventions will confer protective effects in these conditions. In this regard, sustained protective immunity against both murine CL and VL has been achieved by DNA vaccines encoding antigen candidates (Gurunathan et al. 2000, Mendez et al. 2001, Campos-Neto et al. 2002, Zanin et al. 2007, Dondji et al. 2008) or leishmanial recombinant protein(s) formulated with improved vaccine adjuvants (Pashine et al. 2005), including cytosine phosphate guanosine oligodeoxynucleotides, CpG ODN (Rhee et al. 2002, Iborra et al. 2005) and cationic distearoyl phosphatidylcholine (DSPC) liposomes (Bhowmick et al. 2007). Of note, long-term immunity elicited by those vaccines corresponded to, in addition to the presence of leishmania-specific Th1, $CD8^+$ T-cells responses (Gurunathan et al. 2000, Rhee et al. 2002, Sharma et al. 2006). Additionally, heterologous prime-boost vaccination regimes, such as combining DNA priming with a live vectored boost (Gonzalo et al. 2002, Ramiro et al. 2003), or two different live vectors to prime and boost a response (Dondji et al. 2005, Ramos et al. 2008) have been explored as a means of raising protective T-cell responses (Hu 2005).

Due to the genetic variability of human T-cell responses (across HLA haplotypes), T-cell vaccines can elicit variable protective immunity (Robinson & Amara 2005). A second limitation of T-cell vaccines is the potential for T-cells to become exhausted by high levels of persisting antigens (Kostense et al. 2002). Another challenge is the ability of leishmanial parasites to modulate their antigens to evade immune responses (Vanloubbeeck & Jones 2004). Therefore, a successful DNA or subunit protein-based vaccine will likely require a cocktail of proven immunogens. Accordingly, there is increasing emphasis on strategies for combining protective antigen candidates in the same regimen (Campos-Neto et al. 2002, Skeiky et al. 2002, Iborra et al. 2004, Zadeh-Vakili et al. 2004, Moreno et al. 2007, Rodriguez-Cortés et al. 2007, Zanin et al. 2007). It should be noted that a triple fusion protein vaccine (termed Leish-111f-MPL®-SE), consisting of the T-cell adjuvant antigens thiol-specific antioxidant, *Leishmania major* stress-inducible protein 1 and *Leishmania* elongation initiation factor formulated in monophosphoryl lipid A plus squalene, which confers protection in the mouse model against *L. major*, *Leishmania amazonensis* (Coler & Reed 2005) and *Leishmania infantum* infections (Coler et al. 2007) is now within reach. Whether prophylactic immunization using this vaccine can achieve similar levels of immunity against all parasite species that cause disease in genetically diverse human subjects (who also may differ significantly in their nutritional status and previous immunological experience) has yet to be determined.

Additionally, the potential efficacy of the Leish-111f/GM-CSF adjuvant vaccine in a post-exposure paradigm is currently being tested in cases of drug-refractory disease with encouraging results (Badaró et al. 2006). On the other hand, the potential for immunomodulatory factors of sandfly saliva to serve as vaccine targets to prevent pathogen transmission (Titus et al. 2006) has received increased attention by investigators. In this regard, two candidates are the *Lutzomyia longipalpis* salivary gland protein maxadilan (Brodie et al. 2007) and the recombinant protein SP15; a vaccine composed of the latter antigen confers protection in the mouse model against *L. major* challenge infection (Valenzuela et al. 2001).

Natural and experimental leishmanial infections in NHP

Table I summarizes the published studies on natural leishmanial infections in NHP. At least four species of Neotropical monkeys are susceptible to natural infection with human pathogenic *Leishmania (Viannia)* species (Herrer et al. 1973, Lainson et al. 1988, 1989). In contrast, only one species of old world monkeys was found to be naturally infected with *L. major* (Binhazim et al. 1987).

TABLE I
Natural leishmanial infections occurring in Neotropical and old world monkeys

Primate species	*Leishmania* species	Geographic origin	References
Aotus trivirgatus	*L. braziliensis s.l.*	Panama	Herrer et al. (1973)
Saguinus geoffroyi	*L. braziliensis s.l.*	Panama	Herrer et al. (1973)
Cebus apella	*L. shawi*	Brazil	Lainson et al. (1988, 1989)
Chiropotes satanus	*L. shawi*	Brazil	Lainson et al. (1988, 1989)
Cercopithecus aethiops	*L. major*	Kenya	Binhazim et al. (1987)

Monkeys have varying degrees of susceptibility to leishmanial parasites and the specific disease course depends on the challenge parasite (Amaral et al. 1996, 2001, Teva et al. 2003), host species or individual (Dennis et al. 1986, Porrozzi et al. 2006) challenge dose and route of exposure (Lujan et al. 1986a, Amaral et al. 1996). Moreover, sand fly saliva immunomodulators are known to exacerbate leishmanial infection in rodents (Lima & Titus 1996). Accordingly, when rhesus macaques are infected with *L. major* transmitted by *Phlebotomus papatasi* (Probst et al. 2001), they developed skin lesions that lasted longer (12-28 weeks post-infection) than typical infections (11 weeks) induced by needle inoculation with larger numbers (1×10^7) of *L. major* culture metacyclics (Amaral et al. 2001).

Table II summarizes the essential features of the published studies on experimental infection of NHP by various *Leishmania* species. Different NHP species have become useful in studying the biology of infection and in dissecting the host response to these parasites. Those reported as being highly susceptible to *Leishmania donovani* complex parasites include the Neotropical simians *Aotus trivirgatus* (Chapman et al. 1981, Broderson et al. 1986), *Saimiri sciureus* (Chapman & Hanson 1981, Dennis et al. 1985, 1986) and *Callithrix jacchus jacchus* (Marsden et al. 1981). All of these species have since been used as NHP models of VL for anti-leishmanial chemotherapy studies (Dietze et al. 1985, Madindou et al. 1985, Berman et al. 1986). Conversely, East African primates such as Sykes monkeys (*Cercopithecus mitis*) and baboons (*Papio cynocephalus*) all supported low-grade *L. donovani* infections for periods ranging between 4-8 months and subsequently showed evidence of self-cure (Githure et al. 1986). Furthermore, disease mimicking human VL was established in langur monkeys (*Presbytis entellus*) (Dube et al. 1999), vervet monkeys (*Cercopithecus aethiops*) (Binhazim et al. 1993, Gicheru et al. 1995) and macaques (*Macaca mulatta*) (Porrozzi et al. 2006). The *L. donovani*-langur monkey model has also been explored to assess different vaccine formulations against VL (Dube et al. 1998, Misra et al. 2001).

Consistent with documented cases of human CL caused by *L. major*, experimental infection in macaques induced by the same parasite species causes a self-limiting CL of moderate severity (Fig. 1), which resolves within three months (Fig. 2) and provides the most ethically acceptable model for vaccine testing (Amaral et al. 2001, 2002, Campos-Neto et al. 2001). When infected with *L. amazonensis*, macaques developed greater lesion size with longer duration (Amaral et al. 1996). In both experiments, active skin lesions contained amastigotes with a mononuclear infiltrate of macrophages, plasma cells and lymphocytes and formation of tuberculoid-type granulomas. In *L. amazonensis*-infected macaques it was demonstrated that $CD4^+/CD8^+$ T-cell ratios favour $CD8^+$ cells in both active and healing skin lesions (Amaral et al. 2000). A more marked variation in the clinical course of infection was found when groups of macaques were inoculated with different *Leishmania braziliensis* strains (Teva et al. 2003, Souza-Lemos et al. 2008). The inocula produced lesions of variable severity, ranging from localized self-healing CL to non-healing disease (Figs 3A, C). Pathological findings included a typical cell-mediated immunity-induced granulomatous reaction (Fig. 3D), which consisted of all cell types found within human granulomas, including the presence of both IFN-γ- or TNF-α-producing $CD4^+$ and $CD8^+$ T-cells, as well as IL-10-producing $CD4^+CD25^+$ T-cells (Souza-Lemos et al. 2008). While several groups have described that ML (mucosa lesions) has not been observed in Neotropical monkey models of CL (Lainson & Shaw 1977, Lujan et al. 1986a, 1990, Cuba Cuba et al. 1990), in our own studies (Teva et al. 2003, G Grimaldi Jr, unpublished data) two of 30 (6.7%) *L. braziliensis*-infected macaques developed nasal ML (Fig. 3C). In the original model description (Marques da Cunha 1944), ML was observed in two of seven (28.5%) monkeys infected with *L. braziliensis*. Of note, therapeutic responses of *L. braziliensis*-infected macaques to the reference drug *N*-methylglucamine antimoniate (Glucantime®) were consistent with those reported in human disease (Teva et al. 2005).

Contrary to the traditional belief that human self-resolution of CL confers life-long immunity against further infection by the same parasite (Gunders 1987), Killick-Kendrick et al. (1985) and Saraiva et al. (1990) provided evidence that immunity conferred by prior self-resolving leishmanial infection may not always be complete in humans. Likewise, in *L. amazonensis* (Amaral et al. 1996) or *L. major*-infected out-bred macaques (Amaral et al. 2001) both the level of resistance and the acquired immune response to subsequent homologous challenge(s) are variable. The mechanism causing partial protection in primates is not yet clear, but may be related to differ-

TABLE II

Summary of published studies of experimental leishmaniasis in non-human primates, 1927-2008

Host species, number[a]	*Leishmania* species, strain(s)	Challenge route, inoculum dose/source	Disease outcome, course	References
Neotropical simian species				
Cebus apella apella, 7	*L. amazonensis*, PH8	id, 2 x 10^6 prom	Self-limiting CL, 3 mos PI	Garcez et al. (2002)
Cebus apella apella, 6	*L. amazonensis*, H6	id, (?) ama	Self-limiting CL, 9 mos PI	Lainson & Shaw (1977)
Cebus apella apella, 6	*L. mexicana*, M379	id, (?) ama	Self-limiting CL, 6 mos PI	Silveira et al. (1990)
Cebus apella apella, 6	*L. braziliensis*, M1287	id, (?) ama	Self-limiting CL, 8 mos PI	
Cebus apella apella, 6	*L. guyanensis*, M2061	id, (?) ama	Self-limiting CL, 24 mos PI	
Cebus apella apella, 5	*L. lainsoni*, M6426	id, 3 x 10^6 prom	Self-limiting CL, 4 mos PI	Silveira et al. (1989)
Cebus nigrivittatus, 1	*L. infantum*, LEM497	sc, 10^6 prom	Fulminating VL, 58 days PI	Vouldoukis et al. (1986)
Aotus trivirgatus, 2	*L. braziliensis s.l.*, NM8478	id, 10^7 prom	Self-limiting CL, 3.5-8.5 mos PI	Christensen & Vasques (1981)
Aotus trivirgatus, 2	*L. mexicana*, NM8943	id, 10^7 prom	Self-limiting CL, 3.5-6 mos PI	
Aotus trivirgatus, 3	*L. panamensis*, WR128	id, 2 x 10^7 prom	Self-limiting CL, 17-22 wks PI	Lujan et al. (1986a, b)
Aotus trivirgatus, 6	*L. panamensis*, WR128	id, 5 x 10^5 ama	Self-limiting CL, 7-13 wks PI	
Aotus trivirgatus, 8	*L. panamensis*, WR539	id, 2 x 10^7 prom	Self-limiting CL, 20-36 wks PI	
Aotus trivirgatus, 2	*L. donovani*, WR378	iv, 32.5 x 10^6 ama	Fulminating VL, < 25 days PI	Chapman et al. (1981)
Aotus trivirgatus, 6	*L. donovani*, WR378	iv, 32.5 x 10^6 ama	Fulminating VL, > 93 days PI	Broderson et al. (1986)
Saimiri sciureus, 8	*L. braziliensis*, LTB300	id, 5 x 10^{5-6} prom	Self-limiting CL, 6 mos PI	Pung & Kuhn (1987)
Saimiri sciureus, 2	*L. donovani*, WR378	iv, 32.5 x 10^6 ama	Fulminating VL, 41-52 days PI	Chapman & Hanson (1981)
Saimiri sciureus, 12	*L. donovani*, WR378 P/MAR	iv, 32.5 x 10^6 ama kg^{-1} bw	Self-healing VL, 8-13 wks PI	Madindou et al. (1985)
Saimiri sciureus, 7	*L. donovani*, WR378	iv, 5 x 10^7 ama kg^{-1} bw	Fulminating VL, 39-59 days PI (2) Self-healing VL, 8-15 wks PI (5)	Dennis et al. (1985)
Callithrix penicillata, 12	*L. braziliensis*, LTB300, LTB12, LTB179, LTB250	id, (?) ama	Self-limiting CL, 13-25 wks PI (3) Non-curing CL, 15-75 wks PI (9)	Cuba-Cuba et al. (1990)
Callithrix penicillata, 1	*L. amazonensis*, LTB16	id, 2 x 10^7 prom	Self-limiting CL, 6 mos PI	
Callithrix jacchus jacchus, 5	*L. chagasi*, Emperatriz	ip, (?) ama	Persistent VL, > 600 days PI	Marsden et al. (1981)
Old world monkeys				
Macaca fascicularis, 11	*L. tropica s.l.*, (?)	id, (?) ama	Self-limiting CL, 4 mos PI	Parrot et al. (1927)
Macaca mulatta, 11	*L. tropica*, yotvata	id, (?) ama	Self-limiting CL, 14-18 wks PI	Wolf (1976)
Macaca mulatta, 7	*L. major*, LV39	id, 10^7 prom	Self-limiting CL, 3 mos PI	Amaral et al. (2001)
Macaca mulatta, 7	*L. major*, WR1075	sb, (?) prom	Self-limiting CL, 3-7 mos PI	Probst et al. (2001)
Macaca mulatta, 9	*L. major*, Friedlin (V1)	id, 10^{4-7} prom	Self-limiting CL, 10 wks PI	Freidag et al. (2003)
Macaca mulatta, 7	*L. mexicana*, L11 (M379)	id, (?) ama	Self-limiting CL, 3 mos PI	Lainson & Bray (1966)
Macaca mulatta, 4	*L. braziliensis*, L1	id, (?) ama	Self-limiting CL, 3-6 mos PI	
Macaca mulatta, 6	*L. amazonensis*, LTB0016	id, 10^8 prom	Self-limiting CL, 13-25 wks PI	Amaral et al. (1996)
Macaca mulatta, 1	*L. chagasi*, Teves	ip, (?) prom	Persistent VL, 9 mos PI	Marques da Cunha (1938)
Macaca mulatta, 6	*L. infantum*, LV1669	iv, 2 x 10^8 ama kg^{-1} bw	Persistent VL, 60 wks PI	Porrozzi et al. (2006)
Macaca mulatta, 7	*L. braziliensis s.l.*, Henrique	id, (?) prom	Self-limiting CL, 3-8 mos PI (5) Non-curing MCL, > 3-6 mos PI (2)	Marques da Cunha (1944)

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Host species, number[a]	Leishmania species, strain(s)	inoculum dose/source	Disease outcome, course	References
Macaca mulatta, 4	*L. braziliensis*	id, (?) ama	Self-limiting CL, wks PI	Lainson & Shaw (1966)
Macaca mulatta, 10	*L. braziliensis*, SIS and OSC	id, 10^7 prom	Self-limiting (3) and non-curing CL or MCL (7), 14-33 mos PI	Teva et al. (2003)
Macaca mulatta, 9	*L. braziliensis*, SIS	id, 10^7 prom	Self-limiting CL, 16-24 wks PI	Souza-Lemos et al. (2008)
Cercopithecus aethiops, 2	*L. major*, NLB144	id, 10^7 prom	Self-limiting CL, 3 mos PI	Ghiture et al. (1987)
Cercopithecus aethiops, 6	*L. major*, NLB144	sc, 2 x 10^6 prom	Self-limiting CL, < 173 days PI	Lawyer et al. (1990)
		sb, (?) prom	Self-limiting CL, < 92 days PI	
Cercopithecus aethiops, 4	*L. donovani*, NLB065	iv, ip, id, 3 x 10^7 ama plus 3 x 10^7 prom	Self-limiting VL, 4-8 mos PI	Ghiture et al. (1986)
Cercopithecus aethiops, 5	*L. donovani*, NLB065	iv, 10^7 ama kg^{-1} bw	Persistent VL, > 12 wks PI	Binhazim et al. (1993)
Cercopithecus aethiops, 5	*L. infantum*, NLB1495	iv, 10^7 ama kg^{-1} bw	Persistent VL, > 12 wks PI	
Cercopithecus aethiops, 9	*L. donovani*, NLB065	iv, 8 x 10^7 prom	Persistent VL, > 18-36 mos PI	Gicheru et al. (1995)
Cercopithecus mitis, 4	*L. major*, NLB144	id, 10^7 prom	Self-limiting CL, 3 mos PI	Ghiture et al. (1987)
Cercopithecus mitis, 4	*L. donovani*, NLB065	iv, ip, and id, 3 x 10^7 ama plus 3 x 10^7 prom	Self-limiting VL, 4-8 mos PI	Ghiture et al. (1986)
Papio cynocephalus, 2	*L. major*, NLB144	id, 10^7 prom	Self-limiting CL, 3 mos PI	Ghiture et al. (1987)
Papio cynocephalus, 2	*L. donovani*, NLB065	iv, ip, and id, 3 x 10^7 ama plus 3 x 10^7 prom	Self-limiting VL, 4-8 mos PI	Ghiture et al. (1986)
Presbytis entellus, 7	*L. donovani*, Dd8	iv, 10^8 ama	Persistent VL, 105-120 days PI	Anuradha et al. (1992)

a: number of naïve individuals experimentally challenge-infected; ama: amastigotes; bw: body weight; CL: cutaneous leishmaniasis; id: intradermal; ip: intraperitoneal; iv: intravenous; MCL: mucocutaneous leishmaniasis; mos: months; PI: post-infection; prom: promastigotes; sb: sandly bites (either *Phlebotomus papatas* or *Ph. duboscqii*); sc: subcutaneous; VL: visceral leishmaniasis; wks: weeks.

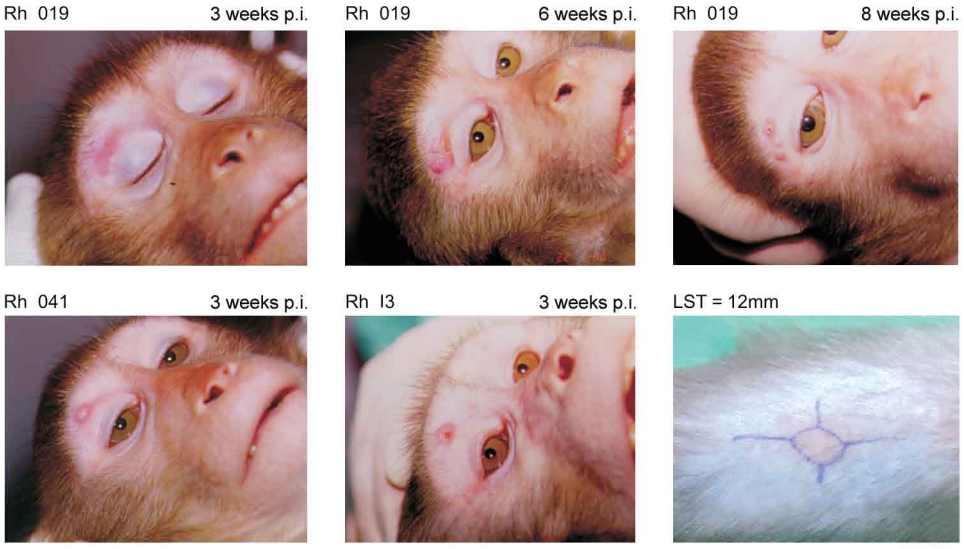

Fig. 1: clinical outcome of self-healing *Leishmania major* cutaneous infection in the primate *Macaca mulatta*. The pictures show the clinical presentations of primary skin lesions observed in monkeys over time post-infection (p.i). In addition, a depiction of a leishmanin skin test-positive reaction detected [rhesus monkey (Rh) 13] is illustrated at week 3 p.i.

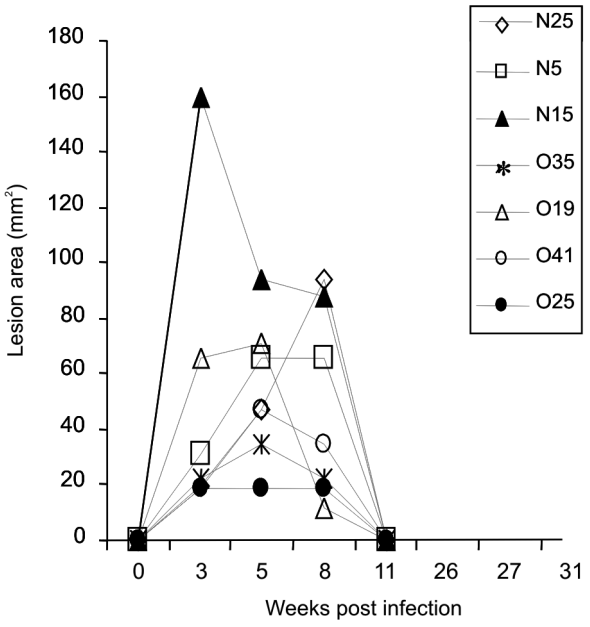

Fig. 2: course of skin lesion development in rhesus monkeys following primary infection with *Leishmania major* (strain LV39). A standardized inoculum of 1 x 10^7 promastigotes (Amaral et al. 1996) was injected intradermally into the orbit of the right eye of each monkey. All of the challenge-infected monkeys developed a typical ulcerated skin lesion at the site of inoculation (Fig. 1). Skin lesions were measured as previously described (Teva et al. 2003).

ential performance of memory T cells (Zaph et al. 2004). In addition, IL-10-producing $CD4^+CD25^+$T cells are known to control acquired immunity in mice (Belkaid et al. 2002) and macaques (Souza-Lemos et al. 2008) with leishmanial infections.

The findings from cross-immunity experiments between different species or strains of *Leishmania* in monkeys (Table III) may give important clues to vaccine reseach. The relative variability in protection after self-cure or drug-cured experimental leishmaniasis to challenge by heterologous parasites appears to reflect both the nature (i.e., etiologic agent) and the course of primary infection or disease tempo (i.e., the progression and resolution of leishmanial lesions). Another factor that can influence acquired immunity is the time between recovery from primary infection and re-challenge. For example, a self-healing CL following infection with *L. major* induces significant protection for *L. amazonensis* and *Leishmania guyanensis* and was dependent on time of re-challenge by *L. amazonensis* after animals had recovered from primary lesions, but lacked protection against *L. braziliensis*. Conversely, macaques immune to either *L. braziliensis* or *Leishmania chagasi* (syn. *L. infantum*) were found to be fully protected to challenge with *L. braziliensis* or *L. amazonensis* (Porrozzi et al. 2004).

All infected animals responded with increased production of immunoglobulins capable of binding to cross-reacting parasite antigens (Lujan et al. 1987, Porrozzi et al. 2004). Although an antigen-specific Th1-like response appears critical for mediating protection in a variety of primate models of CL (Olobo et al. 1992, Olobo & Reid 1993, Amaral et al. 2001, Teva et al. 2003) and VL (Porrozzi et al. 2006), the current parameters of cell-mediated immunity [i.e., by measuring delayed-type hypersensitivity reaction (DHT) to the leishmanin skin test (LST) in vitro lymphocyte proliferation and IFN-γ production] do not always correlate with clinical recovery and resistance to infectious re-challenge (Amaral et al. 2001, Porrozzi et al. 2004, 2006). Certainly, further studying the immune response in primates may clarify what is required to develop and maintain protective immunity to re-challenge(s).

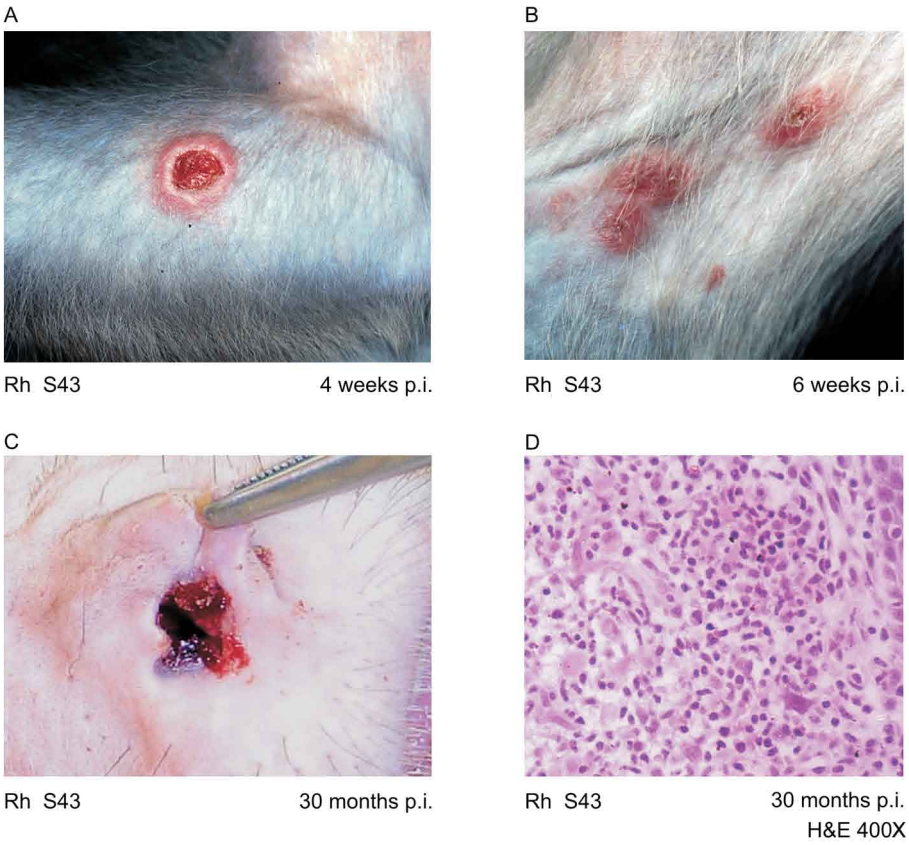

Fig. 3: non-curing *Leishmania braziliensis*-induced cutaneous and mucocutaneous leishmaniasis observed post-infection (p.i.) in a macaque over time. The images show the characteristic clinical features of the disease: the primary ulcerated skin lesion (A); secondary skin lesions (metastases in the extremities) (B); and nasal mucosa granulomata lesion (C). Tuberculoid-type granulomatous reaction (D) was the main histopathological feature of the disease. A standardized inoculum of 1 x 10^7 *L. braziliensis* (strain IOC-L2483) promastigotes (Amaral et al. 1996) was injected intradermally into the left forearm of each monkey.

Use of primate models to assess leishmaniasis vaccines

Divergent evolution (~ 210 million year divergence between rodents and humans) limits the relevance of murine models in guiding the design of human vaccine trials (Nikolich-Žugich 2007). In this regard, old world simian species which diverged from humans approximately 25 million years ago (Gibbs et al. 2007) are emerging as invaluable in vivo models of pathogenesis and immunity to infectious diseases requiring cellular immunity, but are also a key tool for conducting comparative studies of vaccine approaches (Nathansson et al. 1999, Jonhston 2000). Because of the homology between the *M. mulatta* and human immune systems (Kennedy et al. 1997b, Shearer et al. 1999, Pahar et al. 2003, Giavedoni 2005), the NHP model is frequently used to determine which vaccine candidates are most worthy of accelerated development (Johnston 2000, Nikolich-Žugich 2007).

A variety of NHP models for both CL and VL have been used to assess the safety (to verify whether vaccination itself causes adverse effects), immunogenicity (including evaluation of potential correlates of immune protection) and protective efficacy (to determine whether vaccination protects the animal host against infective challenge) of vaccine formulations (Table IV). To date, the only way to determine acquired resistance afforded by a candidate vaccine is to challenge the vaccinated animals with virulent leishmanial parasites. However, because of (i) the limited number of monkeys per experimental group and (ii) the fact that stationary-phase promastigotes can have varying numbers of the infectious form of metacyclic promastigotes within each preparation, researchers use a high inoculum dose to achieve uniform infection for challenge, which may account for the relative variability in the levels of vaccine-induced protection. On the other hand, the use of a short interval between the last boost and the infectious challenge (as short as 3-5 weeks in some studies), makes it difficult to interpret the results in terms of the ability of the vaccine to induce a sustained memory T-cell response (Pitcher et al. 2002). In addition, in most studies of this nature, it is difficult to accurately assess partial host immunity during infection since lesion size, a highly variable parameter (due to the out-bred nature of monkeys used for such studies) is commonly used as a correlate of protection.

The results from primate vaccine studies are summarized in Table IV. Protective efficacy with crude vaccine approaches against CL in macaques was achieved only

TABLE III

Levels of homologous or heterologous immunity to rechallenge infection(s) with *Leishmania* in primates

Species of monkey, number[a]	Challenge infection(s)	Rechallenge infection[b]	Acquired immunity[c]	References
Neotropical simian species				
Cebus apella, 4	*L. amazonensis*, PH8[d]	*L.amazonensis*, PH8	Partial	Garcez et al. (2002)
Cebus apella, 3	*L. mexicana*, L11[d]	*L.amazonensis*, H6	Lack (2), partial	Lainson & Shaw (1977)
Cebus apella, 4	*L. mexicana*, L11[d]	*L.braziliensis*, M1287	Lack (2), partial	
Cebus apella, 5	*L. amazonensis*, H6[d]	*L.braziliensis*, M1287	Lack (1), partial	
Cebus apella, 2	*L. braziliensis*, M1287[d]	*L.amazonensis*, H6	Complete	
Cebus apella, 3	*L. braziliensis*, M1287[d]	*L.guyanensis*, M2061	Lack of protection	
Cebus apella, 1	*L. guyanensis*, M2061[d]	*L.amazonensis* , H6	Lack of protection	
Cebus apella, 2	*L. guyanensis*, M2061[d]	*L.braziliensis*, M1287	Complete	
Cebus apella, 1	*L. mexicana*, L11; *L.braziliensis*, M1287	*L.guyanensis*, M2061	Partial	
Cebus apella, 1	*L. amazonensis*, H6; *L.braziliensis*, M1287	*L.guyanensis*, M2061	Lack of protection	
Cebus apella, 2	*L. mexicana*, L11; *L.amazonensis*, H6	*L.guyanensis*, M2061	Lack of protection	
Cebus apella, 1	*L. braziliensis*, M1287; *L.amazonensis,* H6	*L.guyanensis*, M2061	Partial	
Cebus apella, 1	*L. braziliensis*, M1287; *L.guyanensis*, M2061	*L.amazonensis*, H6	Complete	
Aotus trivirgatus, 3	*L. panamensis*, WR128	*L. panamensis*, WR128	Complete (2), partial	Lujan et al. (1986a)
Saimiri sciureus, 3	*Trypanosoma cruzi*	*L.braziliensis*, WR608	Lack of protection	Pung et al. (1988)
Saimiri sciureus, 3	*L. donovani*, WR378	*L.donovani*, WR378	Complete	Dennis et al. (1986)
Saimiri sciureus, 5	*L. donovani*, WR378[d]	*L.panamensis*, WR539	Lack of protection	Lujan et al. (1990)
Callithrix penicillata, 1	*L. braziliensis*, LTB12	*L. braziliensis*, LTB300	Lack of protection	Cuba-Cuba & Marsden (1992)
Old world monkeys				
Macaca fascicularis, 3	*L. tropica s.l.*	*L.donovani s.l.*	Complete (1) , partial	Ranque et al. (1960)
Macaca fascicularis, 1	*L. infantum s.l.*	*L.donovani s.l.*	Complete	
Macaca fascicularis, 1	*L. infantum s.l.*	*L.tropica s.l.*	Lack of protection	Parrot et al. (1927)
Macaca mulatta, 7	*L. mexicana*, L11 (M379)	*L.mexicana*, L11 (M379)	Complete	Lainson & Bray (1966)
Macaca mulatta, 2	*L. braziliensis*, L1	*L.braziliensis*, L1	Complete	
Macaca mulatta, 2	*L. braziliensis*, L1	*L.braziliensis*, L15	Complete (1), partial	
Macaca mulatta, 6	*L. mexicana*, L11 (M379)	*L.braziliensis*, L1	Complete	
Macaca mulatta, 2	*L. braziliensis*, L1	*L.mexicana*, L11 (M379)	Partial	
Macaca mulatta, 2	*L. mexicana*, L11 (M379)	*L.braziliensis*, L15	Partial	
Macaca mulatta, 3	*L. amazonensis*, LTB0016	*L. amazonensis*, LTB0016	Complete (1), partial	Amaral et al. (1996)
Macaca mulatta, 7	*L. major*, LV39[d]	*L. major*, LV39	Complete (1), partial	Amaral et al. (2001)
Macaca mulatta, 3	*L. major*, WR1075	*L. major*, WR1075	Partial	Probst et al. (2001)
Macaca mulatta, 4	*L. major*, L1	*L. amazonensis*, L2[†]	Complete	Porrozzi et al. (2004)
Macaca mulatta, 9	*L. major*, L1	*L. amazonensis*, L2[‡]	Lack of protection	
Macaca mulatta, 4	*L. major*, L1	*L. guyanensis*, L7	Complete (3), partial	
Macaca mulatta, 6	*L. major*, L1	*L. braziliensis*, L5	Lack of protection	
Macaca mulatta, 4	*L. braziliensis*, L3	*L. braziliensis*, L5	Complete (3), partial	
Macaca mulatta, 3	*L. braziliensis*, L4	*L. braziliensis*, L5	Complete	

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Species of monkey, number[a]	Challenge infection(s)	Rechallenge infection[b]	Acquired immunity[c]	References
Macaca mulatta, 4	*L. chagasi*, L9	*L. braziliensis*, L5	Complete	
Macaca mulatta, 2	*L. major*, L1; *L. amazonensis*, L2	*L. braziliensis*, L6	Lack of protection	
Macaca mulatta, 3	*L. major*, L1; *L. guyanensis*, L7	*L. braziliensis*, L6	Lack of protection	
Macaca mulatta, 2	*L. major*, L1; *L. braziliensis*, L5	*L. braziliensis*, L6	Lack of protection	
Macaca mulatta, 3	*L. braziliensis*, L3; *L. braziliensis*, L5	*L. guyanensis*, L7	Complete (1), partial	
Macaca mulatta, 3	*L. braziliensis*, L4; *L. braziliensis*, L5	*L. amazonensis*, L2	Complete (1), partial	
Macaca mulatta, 3	*L. chagasi*, L9; *L. braziliensis*, L5	*L. amazonensis*, L2	Complete	
Macaca mulatta, 3	*L. braziliensis*, L3; *L. braziliensis*, L5; *L. guyanensis*, L7	*L. panamemsis*, L8	Complete	
Macaca mulatta, 3	*L. braziliensis*, L4; *L. braziliensis*, L5; *L. amazonensis*, L2	*L. guyanensis*, L7	Complete	
Cercopithecus aethiops, 2	*L. major*, NLB144[d]	*L.major*, NLB144[e]	Partial	Githure et al. (1987)
Cercopithecus aethiops, 5	*L. donovani*, NLB065	*L.major*, NLB144	Complete (4), partial	Gicheru et al. (1997)
Cercopithecus mitis, 4	*L. major*, NLB144[d]	*L.major*, NLB144[e]	Complete	Githure et al. (1987)
Papio cynocephalus, 2	*L. major*, NLB144[d]	*L.major*, NLB144 [e]	Complete	Githure et al. (1987)

a: number of animals used in each experiment; *b*: monkeys were rechallenge-infected after they had recovered from previous (primary, secondary and/or tertiary) infection(s). In some experiments, animals were injected with the same parasite strain/dose, but at different time points as indicated (at 28[ǂ] and 44[ǂ] weeks post-infection); *c*: as indicated by the level of clinical resistance to each rechallenge: complete (no lesion), partial (lesion size was smaller and healed faster than in the primary infection) or lack (failure) of protection. In this case, individuals that had recovered from previous infection(s) remained susceptible to the last rechallenge; *d*: host infected twice with the same parasite; *e*: animals were rechallenge-infected nine months after primary lesion resolution.

when the inactivated parasites were combined with alum plus recombinant human IL-12 (Kenney et al. 1999) or CpG ODN (Verthelyi et al. 2002) as adjuvants. In addition, successful vaccination against *L. donovani* visceral infection in langur monkeys was obtained using alum-precipitated autoclaved *L. major* with BCG (Misra et al. 2001). In our previous studies (Amaral et al. 2002) we have compared the potential efficacy of two *L. major* vaccines, one genetically attenuated (*DHFR-TS* deficient organisms), the other inactivated organisms (autoclaved promastigotes with BCG), in protecting macaques against homologous challenge. While a positive antigen-specific recall proliferative response was observed in those vaccinated (79% in attenuated parasite-vaccinated monkeys, versus 75% in ALM-plus-BCG-vaccinated animals), none of these animals exhibited either augmented in vitro INF-γ production or a positive DTH response to the leishmanin skin test prior to challenge. Following challenge, significant differences in blastogenic responses were found between attenuated-vaccinated monkeys and naïve controls. Protective immunity did not follow vaccination, in that monkeys exhibited skin lesions at the site of challenge in all experimental groups. In contrast, vaccination using a mix of the recombinant antigens LmSTI1 and TSA (Webb et al. 1996, 1998) formulated with rhIL-12 and alum as adjuvants induced excellent protection in the high dose *L. major*-macaque model (Campos-Neto et al. 2001). Likewise, vervet monkeys, when immunized with recombinant histone H1 antigen using Montanide as an adjuvant, mounted good protection against challenge with *L. major* (Masina et al. 2003).

Ample evidence supports the notion that different prime-boost vaccination regimens can elicit greater immune responses than single immunization modalities. The use of heterologous prime-boost approaches was originally explored as a means to overcome vector-specific immunity elicited against the priming immunogen and to augment antigen-specific responses by subunit protein boost (Hu et al. 1991). This approach was found to enhance antigen-specific antibody responses in mice, macaques and humans primed with a recombinant vaccinia virus and boosted with recombinant HIV-1 envelope protein (Hu 2005). Conversely, immunization with DNA priming and recombinant virus boosting elicited strong T-cell responses (Schneider et al.1999, Barouch & Letvin 2000). The effect regarding the order of DNA versus recombinant vector for priming or boosting can have in eliciting protective immunity has been debated (Hanke et al. 1998, McClure et al. 2000). Over the past three years, several primate studies have been performed in our laboratory to establish vaccination procedures, improve vaccine immunogenicity and minimize vector-specific immunity. Indeed, it is now clear that detectable *Leishmania*-specific T-cell responses can be induced safely in primates by vaccination, but, depending on the particular regimen used, varying degrees of acquired immunity have been achieved (ranging from non-existent to full protection after the infectious challenge). Further experiments are in progress in the *Leishmania*-macaque model to comparatively examine the potential efficacy of various vaccine approaches afforded by vaccine candidates.

TABLE IV

Summary of pre-clinical trials of prophylactic *Leishmania* vaccine regimens in primates

Species of monkey, number[a]	*Leishmania* vaccine	Vaccination protocol	Challenge infection, inoculum dose, route	Protection conferred by vaccination[b]	References
Macaca fascicularis, 2	HKLV	Monkeys were vaccinated with a single dose of (n = ?) killed *L. tropica* ama, sc	Animals were challenged on week 3 after vaccination with (n = ?) viable *L. tropica* ama , sc	Lack of protection	Parrot et al. (1927)
Macaca mulatta, 12	HKLV	Animals were vaccinated with a single sc dose of killed *L. amazonensis* prom (0.25-1 mg) plus rhIL-12 (2 μg) and alum (0.125-0.5 mg)	Animals were challenged on week 4 after vaccination with 10^7 metacyclic *L. amazonensis* prom, id	Complete	Kenney et al. (1999)
Macaca mulatta, 8	HKLV	Monkeys were primed and boosted (twice), 1 month apart, by injection of ALM (1 mg) mixed with BCG (5 x 10^5 cfu), id	Animals were challenged on week 18 after vaccination with 10^7 viable *L. major* prom, id	Lack of protection	Amaral et al. (2002)
Macaca mulatta, 5	HKLV	Monkeys were primed and boosted, 1 month apart, with 250 μg of GMP-grade HKLV plus 125 μg of alum, combined with 500 μg of a mixture of ODN (D19, D19 and D35), sc	Animals were challenged on week 14 after vaccination with 10^7 viable *L. major* prom, id	Complete	Verthelyi et al. (2002)
Macaca mulatta, 8	LAV	Monkeys were vaccinated with a single dose of 10^8 attenuated *L. major* prom, id	Animals were challenged on week 18 after vaccination with 10^7 viable *L. major* prom, id	Lack of protection	Amaral et al. (2002)
Macaca mulatta, 6	SUPV	Primates were vaccinated twice, 1 month apart, with a mixture of rLmSTI1 (25 μg), TSA (25 μg), rhIL-12 (2 μg) and alum (200 μg). The monkeys were boosted 1 month later with the antigens and alum alone (i.e., without IL-12), id	Animals were challenged on week 5 after vaccination with 10^7 viable *L. major* prom, id	Complete	Campos-Neto et al. (2001)
Cercopithecus aethiops, 8	HKLV	Animals were primed and boosted (twice), 1 month apart, by injection of ALM (1 mg) mixed with rhIL-12 (10 μg), id	Animals were challenged on week 5 after vaccination with 2 x 10^5 viable *L. major* prom plus sandfly salivary gland lysate, id	Partial	Gicheru et al. (2001)
Cercopithecus aethiops, 5	SUPV	Primates received three doses, 2 weeks apart, of rGP63 (50 μg) mixed with BCG (1 mg), id	Animals were challenged on week 4 after vaccination with 2 x 10^5 viable *L. major* prom plus sandfly salivary gland lysate, id	Partial	Olobo et al. (1995)
Cercopithecus aethiops, 7	SUPV	Animals were primed and boosted (twice), 3 weeks apart by injection of rGST-H1 plus MISA720 (200-100 μg for the priming and boosting, respectively), id	Animals were challenged on week 6 after vaccination with 2 x 10^5 viable *L. major* prom plus sandfly salivary gland lysate, id	Complete (1), partial	Masina et al. (2003)

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Species of monkey, number[a]	*Leishmania* vaccine	Vaccination protocol	Challenge infection, inoculum dose, route	Protection conferred by vaccination[b]	References
Prebytis entellus, 8	HKLV	Monkeys were vaccinated with ALM (3 mg) plus BCG (3 mg) using a single or triple dose schedule, id	Animals were challenged on week 8 after vaccination with 10^8 viable *L. donovani* ama, iv	Complete (by triple dosage), partial (by single dose)	Dube et al. (1998), Anuradha et al. (1998)
Prebytis entellus, 8	HKLV	Monkeys received a single dose of alum-precipitated ALM (1 mg) plus BCG (1 mg) in 0.1 mL saline, id	Animals were challenged on week 8 after vaccination with 10^8 viable *L. donovani* ama, iv	Complete	Misra et al. (2001)

a: number of animals vaccinated in each experiment; b: as indicated by the level of clinical resistance after challenge infection: complete (vaccinated monkeys showed early containment of parasite growth in the infected sites and/or developed little or no dermal lesions), partial (lower level of parasite burden in the infected sites and/or skin lesion size was smaller and healed faster in vaccinated monkeys than in control groups) or lack (failure) of protection (the time of skin lesion onset and healing or levels of parasite burden in infected sites were similar in challenged monkeys from either control or vaccinated group); ALM: autoclaved *L. major*; alum: aluminum hydroxide gel (act as adjuvant); ama: amastigotes; BCG: bacillus Calmete-Guerin (used as adjuvant); cfu: colony forming units; CpG ODN: synthetic oligodeoxynucleotides (ODN) containing unmethylated CpG motifs (act as adjuvant); HKLV: heat-killed leishmania vaccine; id: intradermally; iv: intravenously; LAV: live genetically attenuated (*DHFR-TS* deficient organisms) vaccine; MISA720: Montanide ISA 720 (adjuvant); prom: promastigotes; rGP63: a recombinant *L. major* glycoprotein (the major leishmanial surface glycoprotein); rGST-H1: a recombinant glutathione-S-transferase fused to *L. major* histone H1 nuclear antigen (that has no homology to human H1 protein); rhIL-12: recombinant human interleukine-12 (act as adjuvant); rLmSTI1: a recombinant *L. major* homolog of the eukaryotic stress-inducible protein-1 (Webb et al. 1997); rTSA: a recombinant *L. major* homolog of the eukaryotic thiol-specific antioxidant protein (Webb et al. 1998); sb: subcutaneously; sc: subcutaneous; SUPV: subunit protein-based vaccine.

Determining correlates of immune protection to *Leishmania*

While the functional heterogeneity (across HLA haplotypes) of T-cell cytokine responses to existing vaccines is undoubtedly of importance (Robinson & Amara 2005), they have not been extensively analyzed. In fact, T-cell vaccines for microbial infections have been developed without a clear understanding of their mechanism(s) of protection (Lambert et al. 2005). With regard to leishmaniasis, most vaccine studies measure the frequency of IFN-γ-producing Th1 cells as the primary immune correlate of protection (Coller & Reed 2005), but in vitro IFN-γ production as a single immune parameter may not be sufficient to predict protection (Elias et al. 2005, Oliveira et al. 2005). Recent data have shed important insight on the potential correlates of protection, showing that the magnitude, potency and durability of a multifunctional $CD4^+$ Th1-cell cytokine response can be a crucial determinant in whether a vaccine is protective (Darrah et al. 2007). Conversely, it is likely that IL-10-producing $CD4^+CD25^+$ T regulatory cells are functional in determining vaccine failure (Stober et al. 2005). In another study (Stäger et al. 2000), vaccine-induced protection, using the recombinant hydrophilic acylated surface protein B1 (HASPB1) of *L. donovani*, correlates with the presence of rHASPB1-specific, IFN-γ-producing $CD8^+$ T cells.

Neither study in the *L. amazonensis* (Kenney et al. 1999) or *L. major*-macaque model (Campos-Neto et al. 2001, Amaral et al. 2002), nor those in the *L. major*-vervet monkey model (Gicheru et al. 2001), have resulted in a clear definition of what T-cell responses will be required for vaccine-induced protective immunity. Without such knowledge, vaccine design strategies will remain largely empirical, and further failures are likely to occur. This is due, in part, to the high degree of variability in the antigen-specific recall blastogenic and IFN-γ responses detected among primates (Campos-Neto et al. 2001, Pahar et al. 2003). This appears to result from the outbred genetics of macaques used for such studies, which indeed makes them the most appropriate model when predicting the diversity of responses that could be expected in the human population. Increasing the number of monkeys per experimental group can help address this condition. Unfortunately, by definition this is not feasible. On the other hand, using macaques with defined genotypes with respect to immune response genes (MHC class I and II) would minimize individual variability, but unfortunately this approach introduces bias into the results (Johnston 2000, Hu 2005).

Finally, the application of ELIspot and cytokine flow cytometry assays has provided new insights into the attributes of both $CD4^+$ and $CD8^+$ T cells that mediate protection in macaques (Mäkitalo et al. 2002, Keeney et al. 2003). This technology should help to identify correlates of protection in future primate vaccine studies.

Concluding remarks

Clinical development of the available subunit protein or DNA-based vaccines against leishmaniasis may not be fully protective across all HLA haplotypes and *Leishma-*

nia species. This is due, in part, to the inherent difficulties that hinder full characterization of the determinants of successful T-cell immunity in humans (Robinson & Amara 2005, Appay et al. 2008). Nevertheless, most experts believe that a successful *Leishmania* vaccine will likely require (i) selection of a cocktail of protective immunogens; (ii) identification of efficient prime-boost strategies in order to provide broad, cross-reactive and long-lasting protection; and (iii) selection or identification of an adjuvant formulations or delivery systems that can be used in human clinical trials. Nonetheless, given these intrinsic vaccine development requirements, regulatory authorities are willing to regulate safety data on infectious vectored vaccines generated from primates.

However, primate testing should be reserved for the final stages of evaluation of vaccine candidates that have already shown consistent induction of significant protective immunity in conventional mouse models. Considerations for employing the primate *M. mulatta* to evaluate vaccine safety and protective efficacy should also include costs and availability (Kennedy et al. 1997a). Available data indicates that vaccine trials in macaques will not be hindered due to divergence of MHC class I and class II molecules (Watkins et al. 1988, Klein et al. 1993, Doxiadis et al. 2001). Moreover, rhesus macaques have been successfully infected with a variety of human pathogenic *Leishmania* species either by syringe or sandfly challenge and develop a human-like disease (including the non-curing *L. braziliensis* granulomata ML). Most of the published information on the course of primary or secondary infection, clinicopathological changes, immune responses and vaccination data was gained using outbred macaques. Although the predictive value for any animal model in vaccine development ultimately depends on validating data from human trials, further development of the *Leishmania*-macaque model should prove useful in guiding the design of human vaccine trials.

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