

Short communication

INFECTION SITE DEPENDENT PROGRESSION OF CUTANEOUS LESIONS IN AFRICAN GREEN MONKEYS (*CERCOPITHECUS AETHIOPS*) EXPERIMENTALLY INFECTED WITH *LEISHMANIA AETHIOPICA* PROMASTIGOTES

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ABSTRACT: Studies on experimental infection of *Leishmania aethiopica* are very limited due to lack of laboratory animal models. It was previously reported that the African green monkey (grivet monkey) could serve as a non-human primate model of *L. aethiopica* infection. This report provides preliminary data on the development and progression of skin lesions in grivet monkeys when infected with *L. aethiopica* either in the eye lids or the tip of nose. A total of 22 African green monkeys were inoculated subcutaneously on the eye lid (n=14) or the tip of nose (n=8) with 5×10^6 stationary phase promastigotes of *L. aethiopica*. No lesions developed on the eye lids. Lesions of various stages of cutaneous leishmaniasis were observed at the tip of nose. The outcome of *L. aethiopica* infection in African green monkeys and possibly humans could be infection-site dependent. Further studies are needed to examine and understand the immunopathogenetic processes.

Key words/phrases: Cutaneous leishmaniasis, *Leishmania aethiopica*, *Cercopithecus aethiops*

INTRODUCTION

Cutaneous leishmaniasis caused by *Leishmania aethiopica* is an endemic disease in Ethiopia. The disease has three clinical forms: localized cutaneous leishmaniasis (LCL), diffused cutaneous leishmaniasis (DCL) and mucocutaneous leishmaniasis (MCL). LCL is mostly seen as self-healing single lesions in parts of the body not covered by clothing e.g., the face, arms and legs (WHO, 2010). DCL shows multiple lesions on the face, torso and extremities and is usually not self-healing. MCL (Padovese *et al.*, 2009) may result in distortion of nostrils and lips. The most dominant form is LCL; whereas DCL and MCL are less frequent (WHO, 2010).

Animal models are vital in the search of vaccine and drugs for the control of diseases. The availability of an experimental model system for *L. major*, *L. tropica*, *L. infantum* and *L. braziliensis* allowed the explanation of some of the different mechanisms involved in these infections (Latorre-Esteves *et al.*, 2010; Roque *et al.*, 2010; Souza-Lemos *et al.*, 2011; Silva *et al.*, 2012). Non-

human primates such as African green monkeys are also used as an animal model (Gicheru *et al.*, 2009; Mutiso *et al.*, 2012). The utilization of non-human primates susceptible to CL has its advantages because of their phylogenetic closeness to man. Studies on *L. aethiopica* are restricted due to the lack of suitable animal model. Several attempts, carried out to infect variety of laboratory animals (like BALB/c mice, hamster) were not successful (Childs *et al.*, 1984; Humber *et al.*, 1989; Akuffo *et al.*, 1990). Later, Asrat Hailu *et al.* (1995) reported successful infection of grivet monkeys with *L. aethiopica* resulting in cutaneous leishmaniasis.

The aim of this project was identifying the suitable sites for *Leishmania* parasite inoculation in African green monkey to use them for further study for drug and vaccine trials in terms of accessibility and visibility for experimental outcome (for e.g., lesion development). In the previous study the inoculation of leishmania parasite was at the tip of the nose and earlobe (Asrat Hailu *et al.*, 1995). In this experiment, the eye lid was tested as site of leishmania experi-

mental infection since it is easy to observe the lesion in the eye lid while they are in their cage without shaving or catching monkeys. Infection at the tip of the nose is used as the control. It is a comparative study to assess skin lesions development and progression in African green monkeys infected with *L. aethiopicum* at the eye lid and tip of nose.

MATERIALS AND METHODS

Monkeys were trapped from Sodere and Meki (Leishmaniasis non-endemic areas in Central Ethiopia), and held in quarantine for 3 months to exclude natural Leishmania infection, SIV, hemoparasites, and intestinal parasites.

Isolates of *L. aethiopicum* were obtained from LCL and DCL patients after inoculation of dermal scraping lesions in NNN medium. Primary isolates were further passaged into larger culture flasks of the same medium. A portion of the primary isolate was frozen and thawed two weeks before the second phase of infection and put in NNN medium. Third passage stationary phase promastigotes were used for infection at a dose of 5×10^6 as described elsewhere (Rodriguez *et al.*, 2002). The strains were typed as *L. aethiopicum* by isoenzyme electrophoresis.

The infection experiments were set in two phases. In the first phase, 8 monkeys were inoculated with an isolate obtained from a patient with LCL and 6 were inoculated subcutaneously with an isolate obtained from a patient with DCL. In the second phase (after two months of the first infection initiated), 8 monkeys were inoculated subcutaneously at the tip of the nose with the same dose of *L. aethiopicum* promastigotes. In both cases a group of four control animals

were inoculated with uninfected sterile of culture medium (Locks solution: an overlay for NNN medium). Each monkey in the experimental group was followed for two years.

During the follow-up, skin scrapings of lesions of the infected monkeys were taken from the edges of ulcerative lesions and nodules and aseptically transferred to NNN medium (Girginkardesler *et al.*, 2001) and microscopic slide to confirm infection.

The study was approved by the Ethics Review Committee of the former Department of Biology at the College of Natural Sciences, Addis Ababa University (SF/Biol/343/91/99, 1999). Animal trapping was allowed by the Federal and Regional Forest and Wild Life Protection offices of the Ministry of Agriculture and Rural Development of Ethiopian Government.

RESULTS

Leishmania aethiopicum inoculation at the eye lid of 14 African green monkeys did not result in any skin lesions (Table 1). However, the inoculation of similar doses of the promastigotes (5×10^6) at the tip of the noses of 8 monkeys resulted in various stages of skin lesions (nodular and ulcerative) (Table 1). In four of the monkeys, the lesions result in ulcers at the inoculation sites. The uninfected control animals in both groups did not show any lesion development and remained healthy. Promastigotes were observed in NNN cultures medium of skin scraping taken from the ulcerative lesions. However, no parasites were observed in direct smear prepared from skin scraping. Inoculations of isolates obtained from LCL and DCL patients resulted in localized lesions.

Table 1. Clinical manifestation in monkeys experimentally infected with *Leishmania aethiopicum* (*L. aethiopicum*).

Experimental Infection	Type of strain	No of monkeys with different clinical Manifestation		
		Nodules & other clinical sign*	Ulceration	All clinical sign
Phase 1	1 (n=8)	0	0	0
	2 (n=6)	0	0	0
	0 (n=4)	0	0	0
	3 (n=6)	5(50-90)**		8
Phase 2	2 (n=2)	1(50-90)**	3(130-220)**	2
	0 (n=4)	0	1	0

Phase 1= Experimental infection of monkeys with *L. aethiopicum* at the eye lid

Phase 2= Experimental infection of monkeys with *L. aethiopicum* at the tip of nose

Strain type 1 = isolated from patient with localized cutaneous leishmaniasis (LCL)

Strain type 2= isolated from patient with diffused cutaneous leishmaniasis

*other clinical sign=hair lose, redness and indurations

**the number in bracket is numbers of days starting from date of inoculation at which the particular clinical sign appear for the first time.

DISCUSSION

The results of this study are partially consistent with previous report by Asrat Hailu *et al.* (1995). In both studies African green monkeys develop nodular and/or ulcerative lesion in response to inoculation of 5 million *Leishmania aethiopica* at the tip of their nose. However, infection at the eye lid was not included in the previous study so no comparison could be done in this regard.

Leishmania aethiopica infection at the tip of the nose results in nodular and ulcerative lesions within 50–90 and 134–220 days, respectively. This result is similar to reports in humans since cutaneous leishmaniasis caused by *L. aethiopica* gives rise principally to localized cutaneous nodular lesions and ulceration is late or absent (WHO, 2010). Nylen and Eidsmo (2012) indicated that *L. aethiopica* is a parasite associated with slow disease progression and healing. However, there is no clinical sign in African green monkeys inoculated with same strain and similar dose of *L. aethiopica* parasites at the eye lid in contrast to inoculation of the same at the tip nose. This observation, may partially explain that host factor may also contribute in the restriction of *Leishmania aethiopica* infection in few sites of the body even though this is often attributed to the biting behavior of sand flies. Previously, it is also indicated that disease progression is affected by initial site of infection (Yetter *et al.*, 1980).

There could be several possible host related factors that affect the lesion development in different parts of the body. Local immune responses like distribution of immune cells, cytokine profile and immune molecules such as inducible Nitrogen oxide (iNOS) could be one of those reasons. This agrees with the hypothesis that each organ may have a specific immune response (Alexandre-Pires *et al.*, 2010). Therefore importance of local immune response to pathogenesis of *Leishmania* infection is elucidated (Brachelente *et al.*, 2005). As it was reviewed by Nylen and Eidsmo (2012), cutaneous leishmaniasis (CL) is caused by parasitic infection of dermal macrophages resulting in intense immune-mediated tissue inflammation and skin ulceration. Therefore, different disease progression in different parts of the body may be caused by the distribution of immune cells such as macrophages, dendritic cells and mast cells. Previous studies also suggest that clinical course of infection with

Leishmania in humans is associated with specific local patterns of cytokine production (Pirmez *et al.*, 1993, Brachelente *et al.*, 2005). It was also reported that local iNOS expression is a marker of the leishmania resistant phenotype (Cangussu *et al.*, 2009). The distribution of antimicrobial peptides in the skin could also influence cutaneous leishmaniasis disease progression as antimicrobial peptides and proteins (AP) can be induced in inflammatory lesions (Meyer *et al.*, 2007).

Detailed systematic study of the histological, immunological and parasitological changes associated with *L. aethiopica* infection of *C. aethiops* is warranted for better understanding of some of the mechanisms involved in the control of infection in human (Ayele Afshar and Gallo, 2013).

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