# Persistent Infections by *Leishmania (Viannia) braziliensis*

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*Here we review the phenomenon of persistency in* Leishmania (Viannia) braziliensis *infections. In other* Leishmania *species where appropriate animal models exist, considerable advances in the understanding of basic immunologic mechanisms of persistency have been made; for a review see Aebisher (1994). On the contrary, the evidences of persistence in infections with* L. braziliensis *rest on studies of human clinical cases many of which we summarized and discussed in this work.*

Key words: *Leishmania (V.) braziliensis* - persistent infections - American tegumentary leishmaniasis

American tegumentary leishmaniasis (ATL) is a widely spread disease accounting for thousands of new cases per year. Most cases are produced by organisms included in the sub-genus *Viannia* (Lainson & Shaw 1987), with three major species: *Leishmania panamensis* causing single lesions easily cured, with nodules along the lymphatics and few nasopharyngeal lesions; *L. guyanensis* producing disseminate infections compromising the lymphatic system but no nasopharyngeal involvement; and *L. braziliensis* causing primary localized skin ulcers (localized cutaneus leishmaniasis - LCL) with frequent relapses involving the nasopharyngeal mucosa (mucocutaneous leishmaniasis MCL or espundia) a few years later. The cases of leishmaniasis associated with *L. braziliensis* are predominant in many countries leading indefinite follow-ups, extended treatments and hospital bed space occupation for long periods (Marsden 1994).

## PERSISTENCY

A characteristic of all *Leishmania* species is their tendency to establish inapparent infections, or to persist after clinical resolution of the disease. This tendency, well documented and fashionable in the AIDS era (Coura et al. 1987, Bastuji-Garin et al. 1991) was known by the earlier workers in *L. braziliensis* endemic areas. In fact, Lindenberg (1909) reported among workers laying the tracks for the railroad from the State of São Paulo to Mato Grosso, a high incidence of skin ulcers with slow evolution, difficult to cure and with frequent relapses. This picture portrays very well the vicious nature of the infections by *L. braziliensis*.

Different terms have been coined for atypical infections: inapparent or cryptic is accounted for by the presence of any infectious agent with no associated pathology. Persistent infection refers to the permanence of these agents after clinical cure; the infection can stay asymptomatical or show relapses at different times. A persistent infection is also referred to as chronic, but this term is more often applied to a different stage of the disease. For example in hepatitis, ictericia and general weakness remains, and in Chagas' disease, although the parasite is at low numbers, damage to the cardiac muscle is occurring. Chronicity is also related to transmission of a disease by asymptomatic subjects. In the case of *Leishmania*, except for *L. donovani sensus latus*, the transmission between humans appears to be restricted to blood transfusion (André et al. 1957) or organ transplantation (Greemblatt 1980). As the dogma goes in Tropical Medicine, the *Phlebotomus* or *Lutzomyia* need to take a high concentration of parasites which is only attainable at the skin of the animal reservoirs. Despite this, there are reported cases of intradomiciliary transmission that have been attributed to infected *Phlebotomus*, when they bite the parasite-rich wound crest (Rojas & Scorza 1989).

In patients living in endemic areas, it is difficult (if not impossible) to discriminate reactivation (relapse) of a latent infection from a reinfection. There are no appropriate biochemical or molecular markers to distinguish a resident parasite from a newcomer, unless the latter clearly belongs to a different group or population. Karyotype analyses lacks of sensitivity and do not seem to correlate with the zymodeme analysis (Saravia et al. 1990); isoenzymes are useful in discriminating species but lack the sensitivity to distinguish or-

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ganisms of the same population (Saravia et al. 1990), restriction fragment length polymorphism analysis (RFLP) involving unique nuclear genes are in general insensitive, and kinetoplast minicircle restriction analysis analysis (Morel et al. 1980) provide unstable markers, as Pacheco et al. (1995) have shown, variable sequence polymorphisms can emerge during experimental infections with cloned *L. guyanensis* cells. It remain to be tested whether RAPDs analysis targeted in other *Leishmania* sequences can provide the right tool (Macedo et al. 1992).

In a given endemic area it is very difficult to estimate the number of inhabitants with persistent infections. When serological (immunofluorescent antibodies test, IFA, enzyme-linked immunosorbent assay, ELISA) and delayed-type hypersensitivity (DTH) tests are applied in Venezuelan endemic areas, it is frequent to observe a positive response in approximately 12% of the healthy individuals (with no lesions or scars) (Dr Néstor Añez, pers. comm.). Thus, not every person contacting the parasite develops the disease, but unless more sensitive and direct techniques are used, we can not assess whether they have eliminated the parasite.

The existence of cryptic or inapparent infection by *Leishmania* is revealed in patients affected by AIDS when cutaneous or mucocutaneous lesions appear without a previous history of leishmaniasis (Coura et al. 1987, Machado et al. 1992). Recently, using a *L. braziliensis* specific polymerase chain reaction assay (PCR) (Guevara et al. 1992) based on ribosomal nontranscribed spacer sequences, we detected parasite DNA on blood samples of a patient who suffered from multiple cutaneous lesions 30 years ago, and was cured spontaneously (Guevara et al. 1993). Since the patient has lived in a non endemic area ever since, reinfection appears unlikely. An alternative and important source of information about persistent *Leishmania* can be found in individuals who sufferred the disease and latter migrated to non endemic areas or countries where the disease does not exist (Guevara et al. 1994).

A characteristic of *L. (V.) braziliensis* infection is its tendency to metastasis through different parts of the body, with particular preference for the nasopharygeal mucosa. Secondary mucosal infections can occur months or even years after the cure of a LCL. However the parasite can be found in the nasal mucosa without evidences of a primary lesion, or in recent cases of LCL (Villela et al. 1939). It is likely that capacity for metastasis and persistency are closely related; the rapid dissemination of the parasite may improve its chances to find a secure shelter.

## WHY PERSISTENT INFECTIONS?

In Tropical Medicine courses, tegumentary leishmaniasis is thought of as a zoonosis (Manson 1982) where the vertebrate animal host harbors the parasite without apparent harm. According to this view, the human host is an accidental player who suffers the disease as an intruder in the parasite's life cycle. The strong dermal reaction has allergic characteristics, and the cure can occur spontaneously. The picture is more complex because not all humans react with the same intensity or never react. To explain these differences, nutritional factors (Badaró et al. 1986) and genetic susceptibility have been advanced (Lara et al. 1991, Cabrera et al. 1996). Badaró et al. (1986) have implicated malnutrition as an important risk factor for suffering visceral leishmaniasis in children in Brazil. Although this association was found for a viscerotropic parasite, it appears logical that good nutrition is essential to an effective immune system, and the possibility of fighting any kind of infection.

In the mouse, resistance to the infection by *L. mexicana* was shown to be inherited as a dominant character (Pérez et al. 1979) and polymorphic genetic markers have been linked to the susceptibility to leishmaniasis (Blackwell 1988). Some of these markers are also related to the susceptibility to other infections (Roberts et al. 1990). An interesting aspect of these studies is the differential correlation of the genetic markers with different *Leishmania* species (Pérez et al. 1979, Akuffo et al. 1987) a fact that emphasizes the importance of the parasite's genetic makeup in the course of the infection. Because there is no appropriate animal model to study *L. braziliensis* infections, similar studies with this parasite are lacking. The mouse genetic markers are being used as heterologous probes to try to identify «risk» genes in humans. If these polymorphisms are also encountered in human populations, they will open the way for risk assessment and more rational therapy (Alexander & Russell 1992).

In two independent studies searching for genetic markers confering susceptibility to suffer LCL and MCL in Venezuelan families, Lara et al. (1991) working in an endemic area for *L. (V.) braziliensis* and *L. (V.) guyanensis*, have linked the presence of antigens HLA II Bw22 and DQw3, respectively, as genetic risk factors for LCL at the population level. These genetic markers are likely related to the ability of the patients to trigger the TH1 or TH2 arms of the immune response (Gradoni & Gramicia 1994). An experimentally more detailed analysis has been carried out by Cabrera et al. (1996), who analyzed tumor necrosis factor (TNF)-$\alpha$ gene polymorphisms in 25 Venezuelan

patients affected by MCL compared to unaffected controls. In the MCL patients they found a higher frequency of the TNF-308 promoter variant when compared with 43 control subjects. Although it is not clear whether this increase is independent of alleles for human leukocyte antigens (HLA), the authors have suggested a linkage disequilibrium between class II gene and variable genetic elements in the class III region, known to control the TNF-α production as the origin of these associations. In both works, the identity of the parasites was extrapolated from other studies.

Recently, it has been found (Carrera et al. 1996) that when *L. major* promastigotes are incubated with bone-marrow derived macrophages the induction of interleukin 12 (IL12) mRNA and the IL12 release are inhibited. It is likely that the inhibition of the major physiological inducer of interferon gamma (IFN-γ), the most important cytokine for the induction macrophage leishmanicidal action, allows a prolongued survival of the parasite. But also, as has been shown in experimental infections with *L. major*, susceptibility can be determined by the ability of the host to mount a strong TH2-polarized reaction against a restricted set of antigens (Julia et al. 1996).

We do not know whether there is any association between susceptibility to leishmaniasis and the phenomenon of persistence, or whether all the findings in murine models infected with *L. major*, may eventually be valid for human infections with *L. braziliensis* (Da-Cruz et al. 1996), but it seems logical that persistence must reflect a delicate interplay between the parasite and its host. Some *Leishmania* genotypes in a given population, taking into consideration their clonal structure (Tibayrenc et al. 1990), may persist by their capacity to elicit a milder leishmanicidal action, avoiding the exacerbation of the host immune system and causing minor harmful effects to the host.

Alternative explanations have been advanced to try to find a logic for persistent infections, among them premunition defined as a strategy developed by the host to warrant a continuous antigenic production, and thus permanent protection against new infections by the same kind of parasite (Leclerc et al. 1981, Modabber 1987). This hypothesis, although attractive, might be the effect and not the cause. Most likely, as stated above, persistency is reflecting an adaptation of the parasite to propagate without harming the host, allowing the continuation of the infection cycle passing onto the insect. In the case of humans infected by *L. braziliensis* this cycle reaches a dead end when the parasite cannot be taken by the *Lutzomyia*.

In line with this idea, is the mounting epidemiological evidence of totally avirulent strains which do not provoke lesions in immunocompetent individual (Gradoni & Gramicia 1994). Perhaps the continuous destruction of natural environments and reservoirs is pressing the parasite to evolve towards less virulent phenotypes more adjusted to survive in humans.

**WHERE DOES THE PARASITE PERSIST?**

For years, researchers have discussed the possible hideouts of *Leishmania* causing ATL (Ridley 1987); obvious places are the large irrigated organs like spleen or liver, and the lymphatic nodes. In animals infected with *L. braziliensis*, macrophages harboring amastigotes have been observed in spleen trabecules (Drs Lucila Arcay and Elizabeth Bruzual, pers. comm.), and Vexenat et al. (1991) have been able to repeatedly isolate *L. braziliensis* from a chronically infected marmoset. In other dermotropic parasites such as *L. major* (Aebischer et al. 1993) and *L. mexicana* (Arargot de Rossell 1992) parasites have been rescued by culturing organ macerates in immunosupressed animals. It is interesting, that in none of these studies blood was considered a place to search.

The skin is also a place for persistence Schubach et al. (1988) using immunofluorescence techniques, have detected *L. braziliensis* antigens in apparently cured lesions. Moll et al. (1995) have found in experimentally immune mice, dentritic cells harboring persistent *L. major*, these infected dendritic cells are restricted to the lymphatic nodes neighboring the cutaneous lesion. Probably, a similar type of persistence can be found for *L. braziliensis.*

The nasal mucosa, due to its microclimatic characteristics (Marsden 1994), is one of the most attractive places for *L. braziliensis* to stay; this tendency was noticed very early in Brazil by Carini (1911) who reported numerous cases of cutaneous lesions with a late nasal mucosa involvement. The time to develop mucosal leishmaniasis varies from a simultaneous presence of the parasite in the nasal mucosa and skin lesion, to several years after the clinical cure of the skin lesion (Marsden 1994). It would be interesting to check with more sensitive techniques such as PCR, whether patients cured from MCL still harbor *L. braziliensis* in the nasal mucosa.

The possibility that tegumentary *Leishmania* can disseminate, multiply and perpetuate within peripheral blood monocytes has been suggested (Murray 1994). In line with this suggestion, Bowdré et al. (1981) cultured the parasite from the blood of a MCL patient infected in Ecuador, and Ramos et al. (1982) cultured *Leishmania* from peripheral blood cells in cases of LCL and MCL. More recently, Martínez et al. (1992) succeeded

in culturing parasites from peripheral blood in two cases of MCL. In one of the cases, comparing the location of the skin lesion with the nasal infection, these authors suggested haematogenic dissemination. As mentioned above, we were able to detect *L. braziliensis* DNA in blood samples from patients cured many years ago (Guevara et al. 1993) or in asymptomatic subjects coming from endemic areas for *L. braziliensis* (Guevara et al. 1994). In these cases, the higher PCR signal intensities are registered in purified monocytic fraction (Delgado et al. 1996). In patients with recent lesions we rarely detected *L. braziliensis* in blood samples, suggesting that at the beginning of the infection the parasites are restricted mostly to the primary lesion where the insect bite occurred (Guevara et al. 1994); later, when they are being challenged (naturally or artificially), they metastasize and either reach an equilibrium with the host or are eliminated (Padilha-Gonçalves 1988, Guevara et al. 1994). So far, culture of peripheral blood, and PCR have proven to be more efficient in detecting the parasite in blood than fluorescence microscopy analysis of the buffy coat (Cuba-Cuba et al. 1986)

From these observations (Bowdré et al. 1981, Ramos et al. 1982, Martínez et al. 1992, Guevara et al. 1993, 1994) we can suggest that, similar to other dermotropic *Leishmania* peripheral blood monocytes can harbor *L. braziliensis*, and might serve as vehicles for its preservation and development within the human host. Opposed to this tendency, visceral *Leishmania* takes a different course, where the monocytic cells differentiate into macrophages, eventually killing the parasite (Murray 1994).

Finally, although *L. braziliensis* lives in preferred sites within the human host, deviant tropisms have been frequently reported (Padilha-Gonçalves 1988). We believe that inside the extremely complex vertebrate body, and no less complex metabolic and immune systems, the parasite finely adjusts its final destination as a consequence of a permanent and reciprocal interaction with its host.

## PRACTICAL CONSEQUENCES OF PERSISTENCY AND PROSPECTS

If persistency is a common phenomenon in *L. braziliensis* infections, what can be done to limit its impact? In our previous discussion, we have documented the tendency of *L. braziliensis* to persist in immuncompetent subjects with no apparent handicap for the host (Guevara et al. 1994). In these cases it is important to take extreme precautions when the patients are submitted to steroid therapy (Aebischer 1994) or any other immunosuppressing drugs. Also, as a way to control the transmission of the disease in non endemic areas, clinical history and blood screening of blood and organ donors should be enforced. New drugs are being put forward, but so far none of them have surpassed the highly toxic pentavalent antimonials as the drug of choice to keep in check the disease, but not necessarily killing the parasite. Treatments to improve patients' immunocompetency can be of help (Convit et al. 1987), but considering the reports of *L. mexicana* exacerbation in animals after BCG treatment (Grimaldi et al. 1980) better characterized *Leishmania* or BCG fractions are desirable.

Recent studies of the metabolic routes of *Leishmania* may in the future provide more specific and effective drugs to eliminate the parasite. Also, new hopes of controlling leishmaniasis and other infections are arising through a better understanding of how the immune response is modulated. For example, enhancement of the host TH1 response by increasing the IL12 production (Carrera et al. 1996), or tolerogenic approaches for down-regulation of deleterious immune responses (Julia et al. 1996), can provide clean and specific long term protection against infectious agents.

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