

REVIEW ARTICLE

Ecological and evolutionary pressures on leishmanial parasites*

Jeffrey Shaw^{1,2}

A better understanding of the evolutionary pressures experienced by leishmanial parasites can be gained by first considering the evidence used to determine their phylogeny. Ancient phlebotomines of the genus *Phlebotomites* appear to predate the period in which the mammalian orders became separated, by some 40 million years, which lends support to the hypothesis that *Leishmania* evolved from monogenetic insect flagellates. This thesis is further supported by small subunit ribosomal RNAs data analysis that indicates that the *Leishmania* diverged from a trypanosomatid line of monogenetic insect parasites (Fernandes *et al.*, 1993).

Phylogeny of the *Leishmania*

Fossil ancestors of the modern sand flies have been found in Russia and England in sediments of the Jurassic period (160 million years ago) and ancient phlebotomines, which Hennig (1972) considered to be the ancestors of the Phlebotominae *sensu strictu*, were recorded in Lower Cretaceous deposits (120 million years old) in Lebanon. Stuckenberg (1975) noted that the short 5th palpal segment of these ancient African forms is a common feature of many American sand flies. Hennig suggested that there was migration in both directions, over an Afro-South American land bridge that existed beyond the Lower Cretaceous period. Unfortunately after this there is a 100 million year gap

in the fossil records of phlebotomine sand flies till the next specimens were found in amber of the Eocene period (20 million years ago) (Muenier, 1905; Fairchild, 1955).

At the time that the sand flies appeared in the Jurassic period dinosaurs had been the predominant vertebrate fauna for many millions of years, and the first mammals had begun to appear. There is therefore no strong evidence linking the sand fly lineage with the ancient cold blooded vertebrate groups. In this respect it is interesting to note that there are no *Leishmania* in birds, which could be a reflection of the lack of an association between sand flies and the ancestral birds that descended directly from dinosaurs. Likewise the records of *Leishmania* in cold blooded vertebrates are limited to a few species in Old World lizards. The absence or rarity of *Leishmania* in the older groups of vertebrates suggests that the haematophagous habit of sand flies, that are the only known vectors of *Leishmania*, is primarily associated with mammals. Today there are sand flies that preferentially feed on birds, lizards and bats, some of which are associated with the transmission of trypanosomes. Such cases may represent groups that changed their mammalian feeding habits. There are sand flies and marsupials in Australia and it is postulated that there was an interchange between this subcontinent and South America during the Jurassic period. There are, however, no *Leishmania* in Australasia, which became isolated well before Africa and South America separated.

From the above facts it would seem that monogenetic parasites of sand flies adapted to mammals some 90 million years ago, giving rise to the *Leishmania*, during a period when the mammals were diversifying into different orders and when the separation of Africa and South America occurred.

If we consider the ancestral intestinal parasites of sand flies as being monophyletic then *Leishmania* is

* Conference presented at the 42nd National Congress of Genetics, Caxambu, MG, Brazil, September 4-7, 1996.

¹ Departamento de Parasitologia, Instituto de Ciências Biomédicas, Av. Lineu Prestes 1374, Universidade de São Paulo, 05508-900 São Paulo, SP, Brasil.

² Departamento de Parasitologia, Instituto Evandro Chagas, Av. Almirante Barroso, 492, 66090-000 Belém, PA, Brasil. E-mail: jshaw@tba.com.br.

also a monophyletic group. However, adaptation to different mammalian orders could have occurred on different occasions.

The genetic distance between New World *Leishmania* of the subgenus *Viannia* and Old World *Leishmania* of the subgenus *Leishmania* is of the same order as that of mammalian orders that separated some 85 million years ago, which is approximately the time that Africa and South America separated (Tarling, 1980). The absence of leishmanial infections in New World lizards leads me to conclude that the Old World lizard *Leishmania* either represent a mammalian line that became secondarily adapted to lizards or a sand fly line that became adapted to lizards after the separation of Africa and South America. The phylogenetic proximity of lizard *Leishmania* to others has been a point of discussion (Simpson and Holz, 1988; Gomez-Eichelmann *et al.*, 1988; Briones *et al.*, 1992). More recently, using RNA polymerase II gene sequences, Croan and Ellis (1996) observed that two lizard *Leishmania* clustered with mammalian *Leishmania*, forming a clade the divergence of which was comparable to that of *Viannia*. The suggested phylogeny of the present day *Sauroleishmania* mentioned above does not support the idea that mammalian *Leishmania* evolved from those of cold blooded vertebrates.

Further evidence suggesting that *Leishmania* evolved later than some other trypanosomatid groups comes from studies on mitochondrial RNA editing. It is concluded that pan-editing of the whole gene, as seen in such parasites as *Trypanosoma brucei*, is a primitive character and that editing limited to the 5' terminal domain, as seen in *Leishmania*, represents a replacement of this character with partially edited genes (Maslov *et al.*, 1994).

Taxonomic relationships

The *Leishmania* are protozoan parasites that possess an extranuclear DNA body called kinetoplast. It is postulated that this organelle evolved from a bacterial-like organism that invaded the basic stock. Kinetoplastid parasites are morphologically all very similar and follow the same simple pattern of a cell with a nucleus and a single flagellum that originates in the region of the kinetoplast. They have, however, exploited many different strategies to survive and as a result they are physiologically and biochemically a very heterogeneous group of great antiquity. Recent nuclear DNA studies suggest that the *Leishmania* diverged more recently, some 90 million years ago, together with another genus, *Endotrypanum*, from a group that gave

rise to the monoxenous (one host) insect parasites, such as *Blastocrithidia*, *Crithidia*, and *Herpetomonas* (Fernandes *et al.*, 1993). Other kinetoplastid parasites, that branched off earlier, such as the different species of trypanosomes, have two hosts (digenetic).

Although the above mentioned genera are all presently classified within the family Trypanosomatidae, the genetic distances between them are very great. For instance the differences between the genera *Leishmania* and *Crithidia* are of the same order as those between mammals and amphibia. This again reflects conservative morphology contrasted with physiological diversity.

The life cycles of the *Leishmania*

It is impossible to say if the *Leishmania* evolved from digenetic ancestors which had lost their second host, or from monogenetic parasites. Whether or not leishmanial parasitism of mammals is a primary or secondary event it seems, however, that adaptation to different hosts may be a small evolutionary step, involving minimal genetic changes.

One host of a *Leishmania* is its vector, which is a small haematophagus psychodid dipteran insect, known as the sand fly, and the other is a warm- or cold-blooded vertebrate. In their insect host they are extracellular flagellates, known as promastigotes, and in their vertebrate host they are intracellular parasites that have no free flagellum, known as amastigotes. There is evidence suggesting that *Leishmania* survive for life in both hosts, but because the insect's life span is shorter the promastigote phase is correspondingly shorter, especially in areas where sand flies are seasonal.

A key selective pressure was the successful adaptation to a second host in which the parasite could survive for longer periods. Besides adding a greater stability to the population it facilitated contact with other potential vectors, thus allowing the populations to expand.

During their complex life cycle *Leishmania* are exposed to many different extra and intracellular environments and it is here that selection occurs. Perhaps one of their most remarkable accomplishments, however, is that they successfully parasitized the mammalian cells that are responsible for killing invaders - the macrophages. Examples of interactions between host and parasite cell surfaces are perhaps one of the major expressions of plasticity and adaptability, which are critical to the parasites successful survival in two very different micro-environments. Additionally,

however, there are host vector and parasite excreted factors that also can effect parasite survival, and thus genetic selection.

Specificity related to host genetic variability

Different strategies have evolved which result in host specificity. There are examples of host specificity in both vector and reservoir, but the underlying molecular mechanism for these are in general poorly understood. At the ecological level it would seem that vectorial host preferences are a major factor that determines infection with some, but not all the *Leishmania* species in both animal reservoirs and man.

It is presumed that both vector and vertebrate host specificity depends on an interaction between host cell and parasite surface receptors. A major question that needs to be addressed is which, if any, of the two hosts exerts the greatest selective pressure? *Leishmania* are extremely successful parasites and natural infections are found in many different orders of mammals (Lainson and Shaw, 1987). A *Leishmania* is far more likely to become successfully adapted to another reservoir host which the vector feeds on regularly. However, man is susceptible to infection with many species of *Leishmania* whose natural hosts range from edentates to rodents (Lainson *et al.*, 1994), but he is not bitten regularly by the vector, nor is he involved in the enzootic cycle. Likewise the golden hamster (*Mesocricetus auratus*) is not a natural reservoir of any *Leishmania* but it is susceptible to most of the species that infect man. This implies that mammalian host specificity may not be as restricted as the data on reservoir infections suggest. There are, however, a few *Leishmania* such as *L. enriettii* that have only been found in guinea pigs, suggesting a higher degree of host specificity in such cases.

This leads me to assume that a major factor controlling the distribution of the different *Leishmania* in mammals, particularly those that infect man, are the biting habits of the vectors. Ecological factors are a major regulatory external force that determines the distribution of both vector and host. There are environmental situations which will favor the expansion of vector or reservoir populations that could either increase the chance of contact with new hosts or increase transmission rates.

Additionally, however, we must consider why some species that feed on reservoir hosts are vectors and others are not. According to the level of innate susceptibility some sand flies may be refractory to infection while within a species all members may not be

equally susceptible. The important quality of any vector is that the parasite can develop metacyclic promastigotes which are infective to mammals. A member of the *walkeri* group, *Lutzomyia carmelinoi*, is completely refractory to infection yet in nature it is associated with dogs infected with *L.(L.) chagasi*. There are sand flies, such as *Lu. longipalpis*, that support the development of many different *Leishmania* under experimental conditions that in nature they never come into contact with (Lainson *et al.*, 1979). This same sand fly as well as other species have transmitted parasites whose natural vectors belong to other series. An example of this is *Lu. furcata* that experimentally transmits *L.(L.) amazonensis* (Ryan *et al.*, 1986).

Lanzaro and Warburg (1995) reviewed the evidence for the genetic variability of phlebotomine sand flies noting that, as one might expect, there was a greater degree of genetic distance between species of a series than between populations of the same species. The distance between sibling species was of a similar order to those of a series. They also drew attention to the work of Wu and Tesh, which showed that it was possible to select lines of *Phlebotomus papatasi* of high and low susceptibility. However, pure lines of each were not obtained after 17 generations, suggesting that this trait is not controlled by a single locus.

Although there are varying degrees of both sand fly and vertebrate host specificity there is more evidence supporting the idea that the vector exerts the greatest selective pressure, both biologically and ecologically. This is also coherent with the fact that *Leishmania* most probably originated from monoxenous parasites of phlebotominae.

Different levels of susceptibility of individuals within a single population have been shown (Shaw, 1981) for *L.(V.) shawi* in experimentally infected *Lutzomyia longipalpis*. The pattern of infections was not uniform and in a total of 26 flies only 13 had heavy infections in their foreguts compatible with transmission. A *Leishmania* isolated from *Lu. tuberculata* develops in the pylorus of *Lu. furcata*, but a strain of *L.(V.) braziliensis* only developed in the ileum of this same species. The biological importance of pyloric infections for *Leishmania* of the subgenus *Viannia* is unknown, but the above observations show different levels of susceptibility within the intestine at the specific level.

Genetic variations within mammal populations have been shown to play a critical role in determining the course of the infection, regardless of the parasite. Much of the work related to understanding the genetics of this has been done with inbred lines of mice that show varying degrees of susceptibility.

Virulence and infectivity

The *Leishmanias* are horizontally transmitted parasites and it is generally accepted that this form of transmission favors the evolution of increased virulence. There are different patterns of parasite virulence in relation to disease in both cutaneous and visceral leishmaniasis. In general the parasites that cause the cutaneous disease in man do not produce disease in their natural reservoir hosts. On the other hand, those responsible for visceral leishmaniasis cause a fatal disease in dogs, though there is evidence that a few recover spontaneously (Courtenay *et al.*, 1994). Recently Ebert and Herre (1996) considered that the evolutionary process that leads to the maintenance of debilitating or fatal disease is offset by other factors that are beneficial to the parasite.

The classical concept of disease has been considered as being a reflection of a new host/parasite association, but this may not always be the case. Ebert and Herre (1996) mention references to experiments indicating that "Novel parasites are on the average, less harmful, less infectious and less fit than the same parasite strain infecting the host it is adapted to". If a reservoir population turnover is large enough to support heavy infections that result in a fatal disease then parasite fitness is not affected deleteriously. There will be an optimal virulence the value of which will shift according to the vector and host population turnovers. However, selection will depend on the presence of suitable genetic characters, thus in the case of visceral leishmaniasis the dog could exert an evolutionary pressure by favoring the selection and maintenance of virulent strains. The occult nature in the wild reservoir hosts of *Leishmania* species that cause the cutaneous disease in man is an example of the selection of avirulent strains. It is possible that the evolution of avirulent or virulent strains in reservoir hosts is linked to the population structure of both vector and reservoir and to the type of infection in the reservoir host. The important phase for the *Leishmania* in the reservoir is not the pathology but the availability of parasites to the vector. Thus a small number of parasites may be available in the skin over a long period in an occult infection or many may be available over a relatively short period in a virulent infection. Which is selected will depend on the biology of the vector, as well as the reproductive structure of the mammal population.

A *Leishmania* can lose its infectivity to a mammal after being cultured *in vitro*. Infectivity and attachment to the sand fly gut wall, which are major biological events, have been linked to small changes in the terminal sugars of the lipophosphoglycan (LPG)

surface coat of promastigotes (Sacks *et al.*, 1995). Other membrane antigens have been shown to play an important role in infectivity and virulence to mammals and are candidates for vaccines (Grimaldi Jr., 1995). Surface enzymes such as GP63 are also associated with infectivity and have been shown to protect mice when given in liposomes (Russel and Alexander, 1988). These are just a few of the many examples showing the importance that cell surface components have in infection.

Other selective pressures

Theoretically *Leishmania* can exert selective evolutionary pressures on each other. One example of this is the cross immunity that exists between the different species (Lainson and Shaw, 1977). Infection of a host with one parasite could modify or protect it against another which would reduce the potential adaptation to a new host. Leishmanial excreted factors of one species can also influence the development of another species. It was shown that culture medium in which *L.(L.) amazonensis* had been grown inhibits the growth of *L.(L.) mexicana in vitro* (Pacheco *et al.*, 1987). Thus the presence of one *Leishmania* species in a sand fly could presumably inhibit the development of a second. This would select against a second less common species becoming adapted to a sand fly that is already a vector of another. In this respect it is interesting to note that in nature one sand fly species is associated with the transmission of only one *Leishmania* species, although more than one vector may be associated with a single parasite.

The structure of the sand fly's intestinal wall and its contents modulate the development of leishmanial promastigotes and all are presumably important as selective pressures. However, those that facilitate transmission, such as saliva, are crucial. Titus and Ribeiro (1988) noted that an extract of *Lu. longipalpis* salivary glands increased the infectiveness of leishmanial promastigotes. The physiological properties of sand fly saliva evolved with the blood sucking habit and traits that facilitated feeding would be selected. As sand flies evolved the enzymes associated with feeding would also be selected for in accordance with the host on which they preferentially fed. There is no comparative data on the structures of the enzymes used by sand flies during feeding, but it has been shown that different sibling species of *Lu. longipalpis* have varying degrees of vasodilatory activity. It has been speculated (Lanzaro and Warburg, 1995) that these differences result in different clinical presentations of visceral leishmaniasis in man. Such

variations must presumably occur between other sand flies, serving as a mechanism that determines whether or not infection will occur in a particular host. In this example the genetic factors involving selection are primarily related to sand fly feeding but they also impose selective pressures on the metacyclic promastigotes.

RNA viral particles have been found in different isolates of neotropical *Leishmania* from the Amazon region (Guilbride *et al.*, 1992). The fact that they occur in strains from a wide geographical area suggests that they have no deleterious effects and could for example be a help to the *Leishmania* by changing some immune response. These viruses are probably transmitted vertically during division in either host. Viruses are common in many reservoir hosts and vectors of neotropical *Leishmanias*, so horizontal transmission may occur. In the case of a reservoir being infected by flagellates of more than one sand fly, which seems to be very likely, there is a greater likelihood of horizontal transmission within the vertebrate host. RNA viruses are obviously potentially important in the evolution of the *Leishmania*, possibly analogous to the bacterium in the ancestral kinetoplastid. However, more studies to determine how these viruses affect the parasite are needed.

CONCLUSION

Leishmania are exposed to a variety of extracellular and intracellular selective pressures in their vertebrate host and different extracellular pressures in the sand fly's intestine. Present day evidence supports the thesis that the *Leishmania* are relatively recent parasites that took the audacious step of parasitizing the very cells of their mammalian host's immune system that kill invaders. This same environment has also been exploited by a few species of virus, fungi and bacteria. Within the cell they camouflage themselves by controlling the contents of the parasitophorous vacuole, turning it into an amiable rather than a lethal milieu. Within the insect's gut they transform into promastigotes whose surface components are modulated according to their function. There is an explosive phase of development within the insect's intestine which is possibly regulated by the parasites themselves rather than their host. This involves a form of social organization within the intestinal lumen in which some forms are sacrificed so that the metacyclics reach the fore gut, where they will be transmitted to their next mammalian host. This is an essential step because if transmission to another vertebrate does not occur within a matter of days the parasite will die with its vector. Ecological factors have a more profound effect on the vector than the mammal-

ian reservoir. I conclude that the present phylogenetic evidence and the biology of the living *Leishmania* strongly suggest that the major selective pressures occur within the sand fly, particularly in relation to differential susceptibility and reservoir host selection.

Publication supported by FAPESP.

REFERENCES

- Briones, M.R.S., Nelson, K., Beverley, S.M., Affonso, H.T., Camargo, E.P. and Floeter-Winter, L.M. (1992). *Leishmania tarentolae* taxonomic relatedness inferred from phylogenetic analysis of the small subunit ribosomal RNA gene. *Mol. Biochem. Parasit.* 53: 121-128.
- Courtenay, O., MacDonald, D.W., Lainson, R., Shaw, J.J. and Dye, C. (1994). Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brasil. *Parasitology* 109: 273-279.
- Croan, D. and Ellis, J. (1996). Phylogenetic relationships between *Leishmania*, *Viannia* and *Sauroleishmania* inferred from comparison of a variable domain with the RNA polymerase II largest subunit gene. *Mol. Biochem. Parasit.* 79: 97-102.
- Ebert, D. and Herre, E.A. (1996). The evolution of parasitic diseases. *Parasit. Today* 12: 96-101.
- Fairchild, G.B. (1955). The relationships and classification of the Phlebotominae (Diptera: Psychodidae). *Ann. Ent. Soc. Amer.* 48: 182-196.
- Fernandes, A.P., Nelson, K. and Beverley, S.M. (1993). Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: Perspectives on the age and origins of parasitism. *Proc. Natl. Acad. Sci. USA* 90: 1608-1612.
- Gomez-Eichelmann, M.C., Holz, G., Beach, D., Simpson, A.M. and Simpson, L. (1988). Comparison of several lizard *Leishmania* species and strains in terms of kinetoplast minicircle and maxicircle DNA sequences, nuclear chromosomes and membrane lipids. *Mol. Biochem. Parasit.* 27: 143-158.
- Grimaldi Jr., G. (1995). Meetings on vaccine studies towards the control of Leishmaniasis. *Mem. Inst. Oswaldo. Cruz.* 90: 553-556.
- Guilbride, L., Myler, P.J. and Stuart, K. (1992). Distribution and sequence divergence of LRV1 viruses among different *Leishmania* species. *Mol. Biochem. Parasit.* 54: 101-104.
- Hennig, W. (1972). Insektenfossilien aus der unteren Kreide. IV. Psychodidae (Phlebotominae), mit einer kritischen Uebersicht über das phylogenetische System der Familie und die bisher beschriebenen Fossilien (Diptera). *Stuttg. Beitr. Naturk.* 241: 1-69.
- Lainson, R. and Shaw, J.J. (1977). Leishmaniasis in Brazil: XII. Observations on the cross immunity in monkeys and man infected with *Leishmania mexicana mexicana*, L. m.

- amazonensis*, *Leishmania braziliensis braziliensis*, *L. b. guyanensis*, *L. b. panamensis*. *J. Trop. Med. Hyg.* 80: 29-35.
- Lainson, R. and Shaw, J.J.** (1987). Evolution, classification and geographical distribution. In: *The Leishmaniasis in Biology and Medicine. Volume I. Biology and Epidemiology* (Peters, W. and Killick-Kendrick, R., eds.). Academic Press Inc., London, pp. 1-120.
- Lainson, R., Ready, P.D. and Shaw, J.J.** (1979). Leishmaniasis in phlebotomid sandflies: VII. On the taxonomic status of *Leishmania peruviana*, causative agent of Peruvian "uta", as indicated by its development in the sandfly *Lutzomyia longipalpis*. *Proc. R. Soc., Ser. B* 206: 307-318.
- Lainson, R., Shaw, J.J., Silveira, F.T., de Souza, A.A.A., Braga, R.R. and Ishikawa, E.A.Y.** (1994). The dermal Leishmaniasis of Brazil, with special reference to the eco-epidemiology of the disease in Amazonia. *Mem. Inst. Oswaldo Cruz* 89: 435-443.
- Lanzaro, G.C. and Warburg, A.** (1995). Genetic variability in phlebotomine sandflies: Possible implications for Leishmaniasis epidemiology. *Parasit. Today* 11: 151-154.
- Maslov, D.A., Avila, H.A., Lake, J.A. and Simpson, L.** (1994). Evolution of RNA editing in kinetoplastid protozoa. *Nature* 368: 345-348.
- Muenier, F.** (1905). Monographie des Psychodidae de l'ambre de la Baltique. *Ann. Hist.-Nat. Mus. Natn. hung.* 3: 235-257.
- Pacheco, R.S., Grimaldi, G.J. and Morel, C.M.** (1987). Inhibition of growth of *Leishmania mexicana mexicana* by *Leishmania mexicana amazonensis* during *in vitro* co-cultivation. *Mem. Inst. Oswaldo Cruz* 82: 537-542.
- Russel, D.G. and Alexander, J.** (1988). Effective immunization against cutaneous leishmaniasis with membrane antigen reconstituted into liposomes. *J. Immunol.* 140: 1274-1279.
- Ryan, L., Lainson, R. and Shaw, J.J.** (1986). The experimental transmission of *Leishmania mexicana amazonensis* Lainson & Shaw, between hamsters by the bite of *Lutzomyia furcata* (Mangabeira). *Trans. Roy. Soc. Trop. Med. Hyg.* 80: 164-165.
- Sacks, D.L., Pimenta, P.F.P., Malcom, J., McConville, J., Schneider, P. and Turco, S.J.** (1995). Stage-specific binding of *Leishmania donovani* to the sand fly vector midgut is regulated by conformational changes in the abundant surface lipophosphoglycan. *J. Exp. Med.* 181: 685-697.
- Shaw, J.J.** (1981). The behaviour of *Endotrypanum schaudinni* (Kinetoplastidae: Trypanosomatidae) in three species of laboratory-bred neotropical sandflies (Diptera: Psychodidae) and its influence on the classification of the genus *Leishmania*. In: *Parasitological Topics A Presentation Volume to P.C.C. Garnham F.R.S. on the occasion of his 80th Birthday 1981* (Canning, E.U., ed.). Society of Protozoologists, Special Publication No. 1, Allen Press Inc., Lawrence, Kansas, pp. 232-241.
- Simpson, L. and Holz, G.** (1988). The status of *Leishmania tarentolae/Trypanosoma platydactyli*. *Parasit. Today* 4: 114-118.
- Stuckenberg, B.R.** (1975). New fossil species of *Phlebotomus* and *Haematopota* in Baltic Amber (Diptera: Psychodidae, Tabinidae). *Ann. Natal Mus.* 22: 455-464.
- Tarling, D.H.** (1980). The geological evolution of South America with special reference to the last 200 million years. In: *Evolutionary Biology of the New World Monkeys and Continental Drift* (Ciochin, R.L. and Charelli, A.B., eds.). Plenum, New York, NY, pp. 1-41.
- Titus, R.G. and Ribeiro, J.M.C.** (1988). Salivary gland lysates from sand fly *Lutzomyia longipalpis* enhances *Leishmania* infectivity. *Science* 239: 1306-1308.