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Advances in PARASITOLOGY

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PREFACE

The opening paper in this volume concerns the intricate interactions between *Leishmania* and antigen-presenting cells of the mammalian host. Jean-Claude Antoine, Eric Prina, Nathalie Courret and Thierry Lang from the Institut Pasteur, Paris provide a detailed overview of how *Leishmania* spp. interact with two cell types, macrophages and dendritic cells, and describe some of the strategies used by *Leishmania* spp. to survive in these inducible or antigen-presenting cells. This is a fascinating account of the complex interactions that can occur between host and parasites. The authors highlight a number of questions and challenges in need of further research.

In the next paper, Andrew Thompson of the University of Melbourne, Australia and Paul T. Monis from the Australian Water Quality Centre, Salisbury consider the variation observed in *Giardia* and the implications for taxonomy and epidemiology. *Giardia* is an intestinal parasite often encountered in humans, which can cause acute or chronic diarrhoea, dehydration, abdominal pain, nausea, vomiting and weight loss. Awareness of the parasite goes back a long time; indeed *Giardia* might have been observed as far back as 1681 by Antonie van Leeuwenhoek. It is interesting to read how the story has unfolded over the years and to appreciate the considerable ongoing debate that has concerned *Giardia* especially relating to the taxonomy, phylogeny and host specificity. The application of new molecular tools for identification and diagnosis are helping to unravel the mysteries of the transmission and host specificity of this parasite. Undoubtedly the findings have relevance to the control of giardiasis. The authors propose that this new information be reflected in the redesignation of several species of *Giardia* described previously.

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Bernard Fried at Lafayette College, Pennsylvania and Thaddeus Graczyk of Johns Hopkins University, both in the USA, continue the series of reviews on echinostomes (previous reviews in volumes 29, 38 and 49 of *Advances in Parasitology*). The 10 species of *Echinostoma* considered in the present review do not include the most important medical or veterinary parasites, although they can play a significant role in causing disease in waterfowl and aquatic mammals. Some species are also widely used as experimental models since the complete life cycles can be conveniently maintained in the laboratory. This has enabled them to be used to help elucidate many aspects of trematode biology including physiological, biochemical, immunological and molecular studies. These aspects, as well as systematic and descriptive studies, are comprehensively reviewed.

Human hookworm infection is extremely common with estimates of over 700 million cases in the tropics and subtropics. Often occurring together with other intestinal helminths, hookworm infection remains an important public health problem. Indeed there has been a gradual realization that the effects of infection are greater than had been assumed in the past. In this review, Simon Brooker from the London School of Hygiene and Tropical Medicine, UK, Jeffrey Bethony from the "René Rachou" Research Centre FIOCRUZ, Brazil and Peter Hotez from The George Washington University, USA provide an extensive overview of current knowledge highlighting recent advances in our understanding of the biology, immunology, epidemiology and public health significance of hookworm infections. It is extremely encouraging that large-scale treatment campaigns are under way around the world and the authors consider the advantages of regular population-based chemotherapy.

Nico Smit, of Rand Afrikaans University in South Africa, and Angela Davies, of Kingston University in the UK, complete the volume with an account of the relatively little-known but fascinating gnathiid isopods. These small crustacea have free-living, non-feeding adults and parasitic juveniles, comprising several larval stages, which feed on the blood and tissue fluids of fishes. Apart from the sometimes considerable pathogenic effects to the fish of this parasitism, at least one genus of gnathiid (*Gnathia*) serves as a vector

of the apicomplexan protozoan *Haemogregarina bigemina*, a widespread parasite of teleosts. Smit and Davies suggest that further investigation of the capacity of gnathiids to act as vectors of other parasitic groups is warranted.

John Baker
Ralph Muller
David Rollinson

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Leishmania spp.: on the Interactions They Establish with Antigen-Presenting Cells of their Mammalian Hosts

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ABSTRACT

Identification of macrophages as host cells for the mammalian stage of *Leishmania* spp. traces back to about 40 years ago, but many questions concerning the ways these parasites establish themselves in these cells, which are endowed with potent innate microbicidal mechanisms, are still unanswered. It is known that microbicidal activities of macrophages can be enhanced or induced by effector T lymphocytes following the presentation of antigens via MHC class I or class II molecules expressed at the macrophage plasma membrane. However,

Leishmania spp. have evolved mechanisms to evade or to interfere with antigen presentation processes, allowing parasites to partially resist these T cell-mediated immune responses. Recently, the presence of *Leishmania* amastigotes within dendritic cells has been reported suggesting that they could also be host cells for these parasites. Dendritic cells have been described as the only cells able to induce the activation of naive T lymphocytes. However, certain *Leishmania* species infect dendritic cells without inducing their maturation and impair the migration of these cells, which could delay the onset of the adaptive immune responses as both processes are required for naive T cell activation. This review examines how *Leishmania* spp. interact with these two cell types, macrophages and dendritic cells, and describes some of the strategies used by *Leishmania* spp. to survive in these inducible or constitutive antigen-presenting cells.

1. INTRODUCTORY REMARKS

1.1. The Life Cycles of *Leishmania* spp.

Leishmania spp. are heteroxenous, digenetic protozoan parasites and as such they live successively in two hosts, namely hematophagous insect vectors known as sand flies and some mammals playing the role of reservoirs, from which these infectious agents can be transmitted to other organisms of the same species or of a different species, including humans (for a review see Peters and Killick-Kendrick, 1987a; Schnur and Greenblatt, 1995). In female sand flies, *Leishmania* spp. exist extracellularly in the lumen of the digestive tract where they adopt a flagellated, elongated promastigote form and go through several differentiation stages. After a differentiation process called metacyclogenesis, promastigotes infective for mammals, termed metacyclic promastigotes, accumulate in the anterior parts of the digestive tract, from where they can be inoculated into the dermis of mammals during a blood meal (Rogers *et al*., 2002). In mammals, *Leishmania* spp. are obligate intracellular parasites. Indeed, after the bite of an infected sand fly, at least some of the injected metacyclics are rapidly engulfed

by resident dermal phagocytic cells or cells rapidly recruited from the epidermis or the blood. During the early stages of the infection, a large part of the cells internalizing parasites appears to be macrophages (MΦs), inside which promastigotes differentiate into egg-shaped amastigotes devoid of the external flagellum. This process takes several days and occurs within organelles named parasitophorous vacuoles (PVs), the morphology of which, and at least certain properties vary with different *Leishmania* species (Antoine *et al*., 1998; Courret *et al*., 2001). The life cycle is completed when a sand fly takes a blood meal on a parasitized mammal. During this process, the vector can be infected by free amastigotes or by infected mammalian cells located in the skin dermis. In the gut of the insect, the amastigotes differentiate rapidly into promastigotes. As an example, the cycle of *L. amazonensis* is presented in Figure 1.

Humans can also be infected by numerous *Leishmania* species, but for most of them they are accidental hosts. About 12 million people distributed in 88 countries are suffering from leishmaniasis in the world, and it is estimated that 2 million new cases arise each year. In Europe, Africa and Asia, *L. donovani*, *L. infantum*, *L. major*, *L. tropica* and *L. aethiopica* are the main species infecting humans, whereas in South and Central America mainly *L. chagasi*, *L. mexicana*, *L. amazonensis*, *L. guyanensis* and *L. braziliensis* are responsible for leishmaniases. According to the *Leishmania* species initiating infection and their genetic/immunologic status, humans can remain asymptomatic or display more or less severe pathologic processes. Four major forms of human leishmaniases have been described: cutaneous, diffuse cutaneous, mucocutaneous and visceral. Cutaneous leishmaniases are generally benign. Parasites develop locally in the skin at the sites where infected sand flies have inoculated metacyclic promastigotes. In contrast, visceral leishmaniases are fatal in the absence of treatment. In these forms, parasites develop mainly in the liver, the spleen and bone marrow (for a review see Peters and Killick-Kendrick, 1987b; Schnur and Greenblatt, 1995).

As to the wild mammalian reservoirs, which in many *Leishmania* life cycles are rodents, they are generally asymptomatic after infection or develop mild pathologies (Lainson and Shaw, 1979).

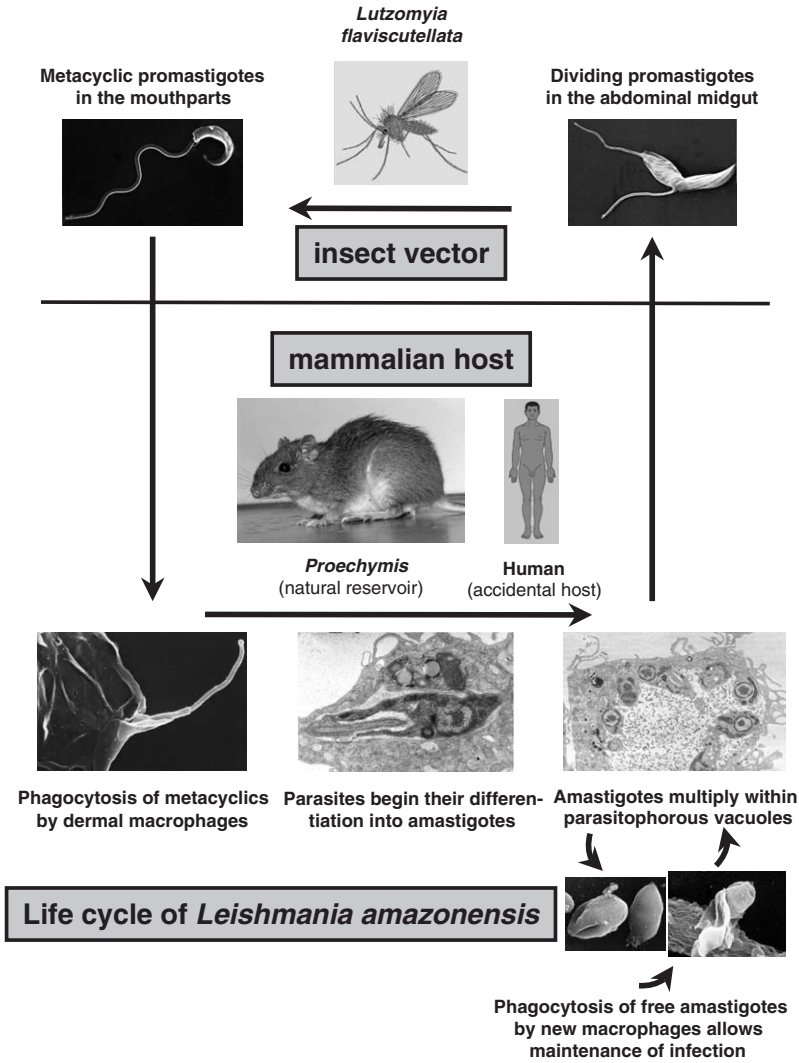

Figure 1 Life cycle of *Leishmania amazonensis*. This *Leishmania* species lives alternatively in the sand fly *Lutzomyia flaviscutellata* and in several rodents, especially in *Proechymis* spp. that are considered as the main reservoirs. *L. amazonensis* is endemic in 17 countries of South and Central America and is present mainly in wet forests of the Amazonian Basin. Humans can be incidentally infected by this *Leishmania* species when they penetrate forest areas but they play no role in the maintenance of the natural transmission cycle. See the text for the description of the different steps of the cycle.

1.2. "Classical and Natural Experimental Models" of Leishmaniases

1.2.1. "Classical experimental models"

The parasite infections due to *Leishmania* spp. have been the object of numerous and detailed studies for at least 15 years. Although mice are not natural hosts for *Leishmania*, inbred laboratory mice inoculated with these parasites have been widely used as experimental hosts for elucidating and characterizing the immunoparasitic processes involved in cutaneous and visceral leishmaniases. However, the "classical models" of leishmaniases are quite different from the situation in nature. They have typically relied on the inoculation of a high number of parasites (10^6–10^8 promastigotes, usually in stationary phase and thus heterogeneous, or sometimes lesion-derived amastigotes, which are not the life cycle stage introduced into the mammalian host by the sand fly bite) into subcutaneous sites (generally the footpad) or intravenously. These protocols are very far from the natural infections where it has been estimated that vector sand flies inoculate 10 to 1000 metacyclic promastigotes into a dermal site (ear, tail, top of the foot).

Host genetic factors controlling both the innate and adaptive immune responses, and consequently the infection outcomes have been discovered using these "classical models". For example, *L. major* parasites induce progressive cutaneous disease in BALB/c mice that are unable to control parasite expansion at the site of inoculation and in the draining lymph node. As a result, BALB/c mice are considered susceptible to *L. major*. In contrast, other strains (C57BL/6 or B10.D2) are considered resistant because the transient lesions that develop at the site of inoculation heal, parasite multiplication is controlled and they develop protective immunity as reflected by resistance to reinfection. However, a low number of amastigotes persist in mice clinically cured and immune to re-infection. Localization of these parasites is still a matter of debate but it seems clear that they are involved in the maintenance of long-lasting immunity. It has clearly been demonstrated that resistance of mice

to primary infection with *L. major* is linked to the IL-12 driven activation/expansion of *Leishmania*-reactive CD4 Th1 cells also known as inflammatory CD4 T cells. This lymphocyte subset produces IFN-γ, which contributes to the activation of macrophages (MΦs) and the nitric oxide (NO) dependent-killing and/or stasis of the intracellular amastigotes. On the other hand, mouse susceptibility to *L. major* has been correlated with the development of a Th2 type response (mediated by CD4 Th2 cells also known as helper CD4 T cells) and the inability to generate a sufficiently potent Th1 type response. In these susceptible mice, the production of cytokines such as IL-4, IL-13, IL-10, TGF-β preventing and/or down regulating the IFN-γ-dependent macrophage activation is thought to participate in sustained disease development (for a review see Reiner and Locksley, 1995; Sacks and Noben-Trauth, 2002).

It is important to stress that the scenario described above does not take into account the role of parasite genetic factors in determining the disease outcome. There is evidence that it does not apply to all *Leishmania* infections and events may differ according to the infecting *Leishmania* species. For instance, most inbred strains of mice, otherwise resistant to *L. major*, are susceptible to *L. amazonensis*, a situation that, for at least some mouse strains, results from an impaired Th1 type response rather than an enhanced Th2 type response (Afonso and Scott, 1993).

Although this point is still being debated, a role for CD8 T lymphocytes in the resolution of primary murine *L. major* or *L. amazonensis* infections has been reported repeatedly (for a review see Milon *et al.*, 1995). After their activation/differentiation, these cells acquire cytotoxic properties but they are also the source of cytokines and especially of IFN-γ.

1.2.2. ''Natural experimental models''

Recently, more ''natural models'' of *Leishmania* infections mimicking as much as possible the natural infections of wild mammals acting as reservoirs were set up in C57BL/6 and BALB/c mice. They rely on the