Drug Resistance in Leishmania Parasites

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Editors

Drug Resistance in Leishmania Parasites

Consequences, Molecular Mechanisms and Possible Treatments

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To Robert (Bob) Killick-Kendrick for his dedication to the study of the disease leishmaniasis, his vast scientific knowledge and great expertise, for his great humor and for being mentor of us all, the leish-maniac family.
To the patients suffering from leishmaniasis, who have helped us to understand this devastating sickness.

Foreword

Therapeutic unresponsiveness presents a formidable obstacle to the efficient clinical management of many diseases. This is especially true in the case of greatly neglected diseases, such as leishmaniasis, for which the drugs currently in clinical use are limited to few antiquated and toxic compounds. The editor of this volume, Alicia Ponte-Sucre, aptly assembled a group of knowledgeable contributors in the field to cover significant aspects of this serious problem. Readers will find in this volume up-to-date information and a fairly complete coverage of the subject. Chapters are well arranged in logical order for better readability. The significance of individual chapters and their organization in this volume warrant discussion.

The introductory chapters in the beginning of this volume provide the necessary background information for those who are unfamiliar with *Leishmania* and leishmaniasis. The editor begins appropriately with the parasite biology and the complexity of leishmaniasis as a disease of different clinical manifestations, i.e., [1] innocuous, self-healing cutaneous lesions; [2] the debilitating diffused and mucocutaneous form; and [3] the often fatal visceral disease or kala-azar. How *Leishmania* has evolved into divergent parasites, which produce such a spectrum disease is a fundamental question of relevance to their inherent phenotype variability, including drug sensitivity. *Gabriele Schoenian*, *Elisa Cupolillo*, *and Isabel Mauricio* pointed out correctly in chapter "Molecular Evolution and Phylogeny of *Leishmania*" that clinico-epidemiologically relevant *Leishmania* need to be collected from all endemic sites to build a comprehensive sequence database for in-depth bioinformatic analyses so that their phylogenetic relationships can be reliably deduced in toto as a foundation to consider their phenotypic differences. Chapter "The Role of Reservoirs: Canine Leishmaniasis" by *Lenea Campino and Carla Maia* discusses succinctly clinico-epidemiology of canine leishmaniasis with emphasis on their resistance to chemotherapy.

With few exceptions, leishmaniasis is indeed epidemiologically a zoonosis. In addition to dogs, the reservoirs include wild canine and rodent species in most places and, additionally, other animals, e.g., sloths, porcupines, and armadillos in South America. Where wild animal reservoirs play a role in the transmission of leishmaniasis, its eradication is virtually impossible to consider. The only option

then is to control this disease that requires rigorous epidemiological surveillance in the endemic sites so that appropriate measures can be implemented to protect the at-risk population from exposure to the sand fly vectors and to the reservoirs, and to treat those already diseased. The question of vector resistance to pesticides and repellents is of relevance to consider, but this is beyond the scope of this volume.

Since neither prophylactic drug nor vaccine is currently available for leishmaniasis, the remaining chapters are mostly concerned with different aspects of drug-resistance in clinical and experimental therapy. Chapter "Epidemiology of Leishmaniasis in the time of Drug Resistance" by *Jean-Claude Dujardin and Saskia Decuypere* addresses the important issue of the regional differences in the epidemiology of drug resistance versus treatment failure. The latter is clinically of unquestionable importance, but does include a variety of logistic issues beyond the main theme of this volume, ranging from manufacture of antileishmanial drugs to their administration and other operational health aspects.

The subsequent immunological components are to the point and necessary for their relevance to therapeutic outcome**.** Chapter "The Role of the Immune System in Resistance to Infection" by *Lukasz Kedzierski and Krystal J. Evans* briefly reviews innate/adoptive immunity to *Leishmania* infection. The authors correctly call attention to the paucity of information on the human immune response to leishmaniasis. Chapters "Vaccination as a Control Measure" and "*Leishmania* Vaccines: Past, Present and Future" by *Katrin Färber & Heidrun Moll and by Bhavana Sethu Lakshmi & Rentala Madhubala* introduce specific and general aspects of vaccination strategy and development, respectively. It is prudent to consider these issues in view of the fact that there is only one peptide vaccine, i.e., Leish-111f+MPL-SE, which has been developed to the stage of clinical trials for immunoprophylaxis of leishmaniasis. These chapters on immunology are very pertinent to the main topic of this volume, considering that immune clearance of the infection is almost certainly necessary for a clinical cure of the disease after chemotherapy, since no drug is expected to reach every parasite in any given patient. Chapter "Co-infection with HIV" by *Margriet den Boer, Luis Rivas & Jorge Alvar* illustrate this point well by alerting readers to the fact that immunosuppression does indeed pose a serious threat to effective therapy of leishmaniasis, as noted from an increase in the incidence of non-healing cases with the spread of HIV-*Leishmania* co-infection.

Immediately following are chapters that outline the specific problems of the topic in clinical and epidemiological terms. In chapter "Visceral Leishmaniasis", *Shyam Sundar and J. Chakravarty* share their invaluable experience in the clinical managements of Indian kala-azar in Bihar. They call attention to the necessity of identifying markers for screening drug-unresponsive patients and to the consideration of multidrug therapy to counter the tenacious problems associated with Sb^V-resistance. Chapter "American Tegumentary Leishmaniasis" by *Olga Zerpa & Alicia Ponte-Sucre* analyzes the challenges we face for a successful diagnosis, treatment and control of American tegumentary leishmaniasis. The incidence of drug resistance increases with expansion of the endemic area in this region. This significant problem is compounded by the epidemiological complexity of the disease due to the diversity of etiological agents and reservoir animals.

The next five chapters summarize the state of our knowledge on the mechanisms of drug resistance based on laboratory discoveries. Leishmanial mechanisms of drug resistance have been elucidated by applying advanced biotechnology. Chapter "Genetic Expression of Drug Resistance, the Role of Proteomics" by *Patricia Cuervo and Jose Batista de Jesus* emphasizes the value of high-throughput proteomic analysis of *Leishmania* and discusses the recent proteomic discoveries of relevance to drug resistance. Chapters "The Role of ABC Transporters in Drug-Resistant *Leishmania*" and "Functional Analysis of *Leishmania* Membrane (Non-ABC) Transporters Involved in Drug Resistance" by *Adriano C. Coelho & Paulo C. Cotrim*, and *Scott Landfear* summarizes laboratory discoveries on the functions of *Leishmania* ABC and non-ABC transporters in drug resistance, respectively. Point mutations of membrane transporters and/or alterations of their expression levels are mentioned to account for drug resistance, as noted in other microorganisms. Chapter "Intracellular Mechanisms of Resistance" by *Mitali Chatterjee* reviews the biochemistry of cellular redox mechanisms in relation to Sb^V-resistance. The significance of cytosolic Sb^V-reductase and other enzymes has been described, consistent with the well-known mechanism of drug conversion by cellular enzymes to modulate the resistance phenotype. The laboratory discoveries described in the foregoing chapters represent significant advances in the basic research of drug resistance, providing molecular targets as its potential biomarkers for evaluation in the clinical samples.

The next few chapters present strategies to develop new drugs and to reverse or alleviate the problem of drug resistance**.** Chapter "Classical Versus Novel Regimens" by *Louis Maes, Raquel Andreia Inocencio da Luz, Paul Cos & Vanessa Yardley* bridges the experimental studies on *Leishmania* in the previous chapters to the next three on drug development for clinical therapy by considering broadly drug sensitivity and resistance in the biological context of host-parasite interactions. In chapter "Drug Targets, Drug Effectors, and Drug Targeting and Delivery" *Phillippe Loiseau and Gillian Barratt* promote the rationale approach to the identification of new targets, and screening natural products for effective antileishmanials. They also describe the strategies of formulation to enhance the effectiveness of drugs in use. Chapter "Mechanisms of Miltefosine Resistance in *Leishmania*" by *Francisco Gamarro, María P. Sánchez-Cañete & Santiago Castanys* focuses specifically on the *Leishmania* mechanisms of resistance to miltefosine via its reduced uptake and efflux, aiming at their characterization for potential use as a clinical marker. Chapter "P-glycoprotein-like Transporters in *Leishmania*: A Search for Reversal Agents" by *Bruno Pradines* discusses the potential and design of flavonoid and other natural "chemosensitizers" to modulate P-glycoproteins as a mechanism to reverse multidrug resistance. The work presented in the foregoing chapters is very pertinent and feasible, calling attention to the necessity of their further development for practical applications.

Development of promising laboratory leads into brand-new antileishmanials which entails a commitment of enormous investments that have been proven too costly to bear by the market force for the unprofitable greatly neglected diseases in general and leishmaniasis in particular. Screening drugs already developed against

other diseases for antileishmanial activities are more cost effective, accounting essentially for the only two therapeutic additions in clinical use for leishmaniasis. Another strategy is to target host molecules in signaling or other pathways, which are modulated by *Leishmania* to ensure their successful parasitism. A renewed interest in this approach is spearheaded by the US-NIH by calling for grant applications to develop drugs in that direction. Such anti-host drugs will inevitably have untoward side effects, but also the potential of their rapid development at a reasonable cost.

Therapeutic strategies to counter the development of drug resistance may include photodynamic therapy, which has been clinically proven and used in treating skin cancer and other diseases. It entails the injection of photosensitizers followed by their excitation with light to rapidly generate a burst of powerful microbicidal ROS. This modality of therapy is unlikely to elicit resistance both in theory and in practice: ROS are known to inactivate multiple targets of cellular molecules with very different properties and the generation of ROS requires a combination of light and photosensitizer, neither of which is cytotoxic in itself. The potential of photodynamic therapy against leishmaniasis has been studied by using, for example, novel endocytic photosensitizers for targeting specificity against the intra-phagolysosomal *Leishmania*. Photodynamically inactivated *Leishmania* also have been explored as a potential carrier to deliver vaccines for immuno-prophylaxis and immunotherapy.

The last two chapters discuss practical and theoretical aspects of the issue at hand. Chapter "The Relevance of Susceptibility Tests, Breakpoints, and Markers" by *Louis Maes, Paul Cos and Simon Croft* points out the need to assess drug sensitivity of different *Leishmania* spp. and to identify molecular markers for resistance under the same conditions by using standardized assays and models. These are very sound proposals, calling for substantial improvements of our ability to duplicate all forms of human Leishmaniasis caused by different *Leishmaia* spp. in the same in vitro and in vivo models. Chapter "The Concept of Fitness and Drug Resistance in *Leishmania*" by *Alicia Ponte-Sucre, Emilia Diaz, and Maritza Padrón-Nieves* introduces the idea that the development of drug resistance by *Leishmania* may change their fitness as a parasite, considering that they are already constantly subjected to stressful conditions throughout their life cycle. This is an intriguing, but complicated, question that may be assessed after full elucidation of the mechanisms of *Leishmania* drug resistance and pathogenicity so that the actual "costs" for the "fitness" of these respective phenotypes can be estimated.

Together, the chapters contained in this volume offer readers not only a comprehensive view of the current status and knowledge of *Leishmania* drug resistance but also point out the future direction of the necessary work toward its resolution. By bringing chapters of laboratory research and clinico-epidemiological concerns together into a single volume, the gap between the two becomes evident, highlighting an urgent need for a closer collaboration between basic scientists and clinicians to foster effective bench-side to bedside translational research.

Kwang-Poo Chang

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About the Editors

Professor Dr. Emilia Díaz

Emilia Díaz studied Pharmacy at the Central University of Venezuela (UCV) in Caracas and became Doctor of Sciences in Pharmacology in 2003 in the same university. From 1999 to 2003, she was Assistant Professor at the Chair of Biology, Faculty of Pharmacy, UCV. Currently she is Associate Professor of Human Physiology, Faculty of Medicine, UCV. Initially her research interests were related to the comprehension of the mechanisms involved in the diuretic and natriuretic action of adrenomedullin, and the role of the dopaminergic system in the adrenomedullin-induced diuresis and natriuresis. Recently, her research interests are focused toward the comprehension of the mechanisms involved in proficiency and fitness in drug-resistant *Leishmania* and in the initial interaction events (chemotaxis) occurring between *Leishmania* and its host cell.

Professor Dr. Maritza Padrón-Nieves

Maritza Padrón-Nieves studied Biology at UCV in Caracas and became Magister Scientiarum in Pharmacology in 1993 and Doctor of Sciences in Pharmacology in 2011 in the same university. From 1994 to 2000, she was head of the Department of Basic Sciences at the School of Nursery, UCV. Currently she is Associate Professor and head of the Human Pharmacology and Toxicology Chair, Faculty of Medicine, UCV. Initially, her research interests were related to the comprehension of the mechanisms involved in digoxin intoxication. Recently, she has devoted her scientific interest in the identification and characterization of molecular markers of resistance in the *Leishmania* sp. infection model.

Professor Dr. Alicia Ponte-Sucre

Alicia Ponte-Sucre studied Education in Biological Sciences at the Andrés Bello Catholic University in Caracas and became Magister Scientiarum in Physiology and Biophysics in 1981 at the Venezuelan Institute of Scientific Research and Doctor of Sciences in Pharmacology in 1993 at the UCV. She spent a year (1999–2000) at the University of Würzburg with a scholarship from the Alexander von Humboldt Foundation and was a staff scientist (2003–2007) at the same university, within a multidisciplinary project from the German Research Council. Currently, she is Full Professor in Human Physiology and Coordinator for Scientific Affairs of the Faculty of Medicine, UCV. Initially, her studies were focused on the physical-chemical behavior of black lipid membranes. Later, her scientific activity was oriented toward the characterization of receptors involved in airway smooth muscle contraction. Recently, her interests have been focused toward the study of parasite metabolism and membrane transporters essential for parasite survival and involved in drug resistance and the mechanisms involved in cellular differentiation and parasite-host interaction in the *Leishmania* model. Additionally, she has characterized natural products and target-oriented designed compounds as potential therapeutic agents.

Introduction: Leishmaniasis – The Biology of a Parasite

A. Ponte-Sucre

Introduction

Leishmaniasis is a disease caused by various species of an obligate intracellular parasite of the genus *Leishmania*. This parasite dwells in cells of the monocytic-phagocytic system of mammals and is transmitted by female sand flies. More than 20 *Leishmania* species are pathogenic to humans, and more than 30 species of sand flies are vectors. The disease is endemic in several world areas, including deserts and rain forests in tropical and subtropical regions of Africa, America and Asia, and sub-rural and urban areas in southern Europe (Davies et al. 2000; Croft et al. 2006; Rotureau 2006; Ready 2010). An estimated 350 million people worldwide are at risk of being infected; about 12 million people are infected, and annual occurrence is about 1.5–2 million cases of the cutaneous and 500,000 cases of the visceral form of the disease (Croft et al. 2006). In anthroponotic foci, sand flies transmit parasites from human to human, and in zoonotic foci, sand flies transmit parasites between the usual local hosts and from them to humans (WHO 2010; Rotuerau 2006). Of note, males are normally more affected than females, especially in sub-Saharan Africa.

Epidemiology

Worldwide, leishmaniasis occurs in 88 countries or territories. Visceral leishmaniasis is found in many of those countries, with most of the estimated annual cases occurring in poorer rural and suburban areas of Africa, America and Asia

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