Alicia Ponte-Sucre
Maritza Padrón-Nieves *Editors*

Drug Resistance in Leishmania Parasites

Consequences, Molecular Mechanisms and Possible Treatments

Second Edition

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To our students, their curiosity inspires us to continue in our path of searching and learning about this ancient disease.
To the patients suffering leishmaniasis, our best partners to understand and fight against this devastating disease.

Foreword

The year 2013 saw the publication of the first edition of the volume *Drug Resistance in Leishmania Parasites*, edited by Alicia Ponte-Sucre. A brand-new edition is now available in which ten of the chapters have been drastically updated, two chapters slightly revised, and two entirely new chapters added. This updated version is timely, because recent years have revealed important new information about drug resistance in *Leishmania.* Furthermore, major efforts are being made to control this problem. Knowledge has increased by studies of the mechanisms by which resistance is generated and by epidemiological and population genomic research on how drug resistance spreads. Moreover, evidence has been accumulating that the relationship between therapeutic failure and drug resistance is complicated; therapeutic failure cannot always be correlated with drug resistance. Current research is therefore being performed to understand the relationship and how both phenomena can be dissected. Furthermore, important achievements have also been made in research to develop tools for diagnosis, treatment, and control of the leishmaniases.

Alicia Ponte-Sucre and Emilia Diaz provide a general overview of the biology of *Leishmania* in the introductory chapter of this volume and highlight the challenges faced in combatting the drug-resistant forms of leishmaniases. This Introduction focuses particularly on aspects of the parasite's biology which are relevant for an understanding of topics covered by the following chapters of the book, each devoted to an important, specific aspect of drug-resistant parasites and the diseases they cause.

The different chapters are grouped into four different parts. The **first part** covers three articles that address "determinant features in leishmaniasis." Gabriele Schönian and colleagues present new insights into the evolution, taxonomy, and phylogenetic and population genetic relationships of *Leishmania*, as acquired by recent research. The power of modern approaches used in the research, such as multilocus sequence analysis, multilocus microsatellite typing, and comparative genomics for studying the inter- and intraspecies variation of *Leishmania* parasites, is discussed. In the following chapter, Lenea Campino and Carla Maia review the epidemiology, pathogenicity, and treatment of leishmaniasis in dogs and the role of dogs as reservoir hosts of *L. (L.) infantum* parasites. Additionally, the role of other mammals as potential reservoir hosts of parasites belonging to the *L. (L.) donovani* complex is addressed. These authors discuss the potential generation and spread of drug

resistance by the use of the same compounds in both canine and human hosts and the measures to be taken to control human zoonotic leishmaniasis.

Jean-Claude Dujardin provides an update on the epidemiology of leishmaniasis in relation to drug-resistant and treatment-failure parasites, whose phenotypes are based on the analysis of parasites in the laboratory and on the clinical assessments of patients, respectively. Whereas in the previous edition he presented results obtained for antimonials, the work has now been extended to miltefosine and the data are compared. Risk factors for (re-)emergence and spreading of leishmaniasis are discussed by focusing on the link between drug-resistant and treatment-failure phenotypes, such as the role of asymptomatic carriers and animals, coinfection with HIV and *Leishmania* RNA viruses, human migrations, and environmental changes. Additionally, the advances made in the development of tools for epidemiological surveillance of treatment failure/drug resistance are described, ranging from clinical tools to laboratory ones. In the last chapter of this section, Lukasz Kedzierski and Krystal Evans review our current knowledge of the immune factors involved in controlling leishmaniasis and discuss the role the immune system plays in resistance to the parasitemia. The parasites have evolved a variety of strategies to evade leishmanicidal mechanisms and survive in the phagosome of macrophages. Whereas most infected individuals develop long-lasting protective immunity following primary infection, sterile immunity is rarely achieved and parasites may persist asymptomatically in the host. The authors describe the vast array of immune cells and cytokines involved in the immune response to the infection which highlights the complexity of the disease and reveals a complicated network of regulatory as well as counter-regulatory interactions that contribute to the persistence.

The **second part** of the book contains four chapters addressing the "challenges in diagnosis, treatment, and control of leishmaniasis in times of drug resistance." Combined infection by HIV and *Leishmania* is a well-known problem. Margriet den Boer and colleagues describe the epidemiology, current spread, clinical aspects, and management of this coinfection. They discuss how development of drug-resistant *Leishmania* strains complicates chemotherapy for *Leishmania*/HIV coinfection and what are the prospects for future chemotherapeutic alternatives which target *Leishmania* and HIV and tackle both infections simultaneously. Shyam Sundar and Jaya Chakravarty describe how, over the years, therapy for visceral leishmaniasis has changed because of the increased unresponsiveness for existing drugs. Whereas pentavalent antimonials have been the mainstay for treatment during most of the twentieth century, a significant subset of patients in the Indian subcontinent were apparently no longer responsive in the 1980s, even when dosage was increased substantially. Unfortunately, there is no marker yet validated for this unresponsiveness, although there are recent reports about cases where IgG1 seemed to be a good predictor of relapse when measured at the end of treatment for visceral leishmaniasis. The authors describe alternative therapeutic options that have been developed, such as conventional amphotericin-B or its lipid formulations, oral miltefosine and paromomycin, and the efficacy and recommended use of these alternative drugs.

Olga Zerpa and colleagues describe the current situation of tegumentary leishmaniasis in the American continent. It is an endemic anthropozoonosis caused by several species of both the *Leishmania* and the *Viannia* subgenera and may thus cause different pathologies. The control of this disease meets with several difficulties: the parasites have several reservoirs and use various vectors to infect humans and mammals. Current treatment involves antimonials, but the efficacy is unpredictable, probably at least in part due to drug resistance. The disease is expanding in the American continent. The authors compare some of these data with those about the disease in the Old World for a better appreciation of the unique aspects of the American tegumentary leishmaniasis. The authors argue that the precise identification of the species of the infectious agent is crucial for correct clinical diagnosis, appropriate treatment, and control of the disease, especially in relation to the challenges imposed by drug resistance. The last chapter of Part II is a new contribution by Guy Caljon and colleagues about the challenges for effective leishmaniasis treatments. The challenges are the important emergence and spread of resistance against the pentavalent antimonials in recent decades, after their successful use for over 70 years in therapy for visceral leishmaniasis, and the huge species- and strain-specific variations in drug susceptibilities that dramatically complicate effective treatment of cutaneous leishmaniasis, although this cannot be linked to development of drug resistance. Moreover, anti-leishmanial treatment failures increasingly occur with all of the currently available standard drugs. The factors probably responsible for these failures, which are related to the complex interplay between parasite, host, and drug, are discussed along with their consequences for therapy and development of new drugs.

Part III comprises three chapters about "molecular features of drug-resistant *Leishmania*." Patricia Cuervo and coworkers stress the importance of proteomic approaches in researching leishmaniasis pathogenesis and problems such as drug resistance. Genomics and transcriptomics studies are important but insufficient to reveal the full picture because posttranscriptional and posttranslational processes play a crucial role in protein expression in these parasites. The genomic data, complemented with high-throughput proteomic analysis, can shed light on resistance mechanisms and identify new drug targets against leishmaniasis. Proteomic analysis of *Leishmania* parasites has already provided information about drug resistance mechanisms. The characterization of the proteins involved has advanced, but still many fundamental questions remain to be answered. Adriano Coelho and Paulo Cotrim summarize research on ABC transporters in membranes of *Leishmania* parasites. Genome sequencing identified in different species of the genus the presence of members of all eight known different subfamilies of ABC transporters, each having specific functional characteristics. The authors discuss the work that revealed how some of these transporters are associated with drug resistance in leishmaniasis and showed their role in the pathology caused by the parasite and how the activity of these proteins affects the efficacy of the treatment. The next chapter deals with non-ABC transporters of *Leishmania* which are responsible for uptake of nutrients by the parasites but may also be exploited for mediating transport of drugs. Such transporters may become responsible for drug resistance of the parasites by

mutations in their coding regions or changed expression. Scott Landfear gives an update on how the analysis of the *Leishmania* genome and recent functional studies have increased our knowledge about different classes of solute transporters involved in drug uptake and how modification of their structure or expression level confers changes in drug sensitivity and causes drug resistance.

The **fourth and last part** of the book is devoted to "tools and strategies to circumvent resistance in *Leishmania*." Shishir Gupta and Thomas Dandekar have added a new chapter in which they describe how bioinformatics is being used for querying genome, transcriptome, and proteome information to identify potential new targets for drug discovery and vaccine development in *Leishmania*. Furthermore, the authors provide information about software used in such research and give links to websites where tools can be found to examine and rank the new targets. Bruno Pradines has updated his chapter about P-glycoprotein-like transporters in *Leishmania*. Drug resistance can be due to different mechanisms that result in decreased level of the drug in the parasite. One of these mechanisms, well recognized as responsible for antimony resistance in *Leishmania,* involves an increased efflux, mediated by P-glycoprotein (Pgp)-like transporters. P-glycoproteins, well characterized in research of drug-resistant cancers, belong to the superfamily of ABC transporters. Inhibition of the drug efflux by these proteins will thus offer an attractive manner to control drug-resistant parasites in a patient. Indeed, the author describes a number of natural or synthetic compounds, some being derivatives known to modulate human Pgp, which are able to revert the resistance phenotype in parasites to a variety of drugs commonly used in both visceral and cutaneous leishmaniasis by decreasing their intracellular concentration. Concepts about the reversal mechanism of multidrug resistance by the use of chemosensitizers which alter the capacity of Pgp are discussed.

In the final chapter of this volume, Manu Vanaerschot and colleagues provide an updated chapter about "the concept of fitness and drug resistance in *Leishmania*." When pathogens develop resistance against drugs, it usually comes at the cost of making them less fit than their wild-type counterparts. This has important implications for the frequency of treatment-failure cases in endemic regions. Cases of treatment failure in patients suffering from leishmaniasis have been observed for most anti-leishmaniasis drugs. However, it is intriguing that this failure could not always be correlated with drug resistance of the infecting parasites, since cases of failure upon treatment with both pentavalent antimonials and miltefosine were accompanied with an increased fitness of the *L. L. donovani* parasites. The authors argue that these examples highlight parasite fitness as a potentially important contributor to treatment failure, at least for visceral leishmaniasis in the Indian subcontinent. They discuss available information and remaining questions about fitness for different *Leishmania* species and the different stages of their life cycle, as well as the relevance of parasite fitness for the development and spread of drug resistance and/or treatment failure in the field, and for new research toward the development of drugs for leishmaniasis and the control and elimination of the disease.

Thanks to the combined efforts of the editor and the authors of the 15 chapters, this book provides an excellent overview. It covers the current stage of our knowledge about the major problems of drug resistance of *Leishmania* parasites, as well as treatment failure in the different manifestations caused by various species of the genus. It presents the current knowledge and questions about the pathology and epidemiology of the leishmaniases in the context of *Leishmania* biology. Diagnosis, treatment, and molecular-parasitological aspects are all discussed from the perspective of drug resistance and how this could be dealt with. This book will therefore be a highly valuable source of information for both basic researchers and clinicians with interests in leishmaniasis.

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Preface

Old and New World leishmaniasis are in urgent need of reevaluation of treatment guidelines as treatment failure is an everyday growing problem. For this disease, treatment failure and drug resistance are topics that go hand in hand. Additionally, field parasites may be naturally resistant to classical drugs or might be selected as resistant by the use of current therapies. These features are (at least partially) responsible for the disappointing picture of disease persistence and death worldwide presented by leishmaniasis. A better understanding of the ailment and of drug resistance, its molecular basis, consequences, and possible treatment may help improving this depressing picture. We hope that this volume will help us to achieve this goal.

The work done by each of the authors and coauthors contributing to this volume has been awe-inspiring. Each chapter is intended to supplement well-documented texts that cover from molecular evolution to the design of compounds that may impact the drug resistance problem, as well as to the comprehension of how adaptable these parasites are. Our intention with this reedited and updated volume is to continue targeting scientists, pre- and postgraduate students, and scholars involved in the medical treatment of patients with leishmaniasis, or dedicated to the design of novel compounds and lead pharmacophores against leishmaniasis.

We acknowledge the Coordination for Research, Faculty of Medicine, and the Council for Scientific and Humanistic Research, Universidad Central de Venezuela, as well as the Missionsärztliche Institut, Würzburg, and the Alexander von Humboldt Foundation, Germany, for their support to our work for so many years. Additionally, we acknowledge the support from the Siebold-Collegium Institute for Advanced Studies, University of Würzburg, Germany, during the last steps of compilation and final organization of the content of this volume. Finally, we would like to address the excellent support of the staff at Springer, Rajeswari Balachandran, Tanja Grabner, and Claudia Panuschka for their guidance through the different steps of the publication of the book.

Caracas, Venezuela — Alicia Ponte-Sucre

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About the Editors

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. During the last 30 years, her interests have been focused on 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 Trypanosomatids, but especially in the *Leishmania* model. Additionally, she has characterized natural products and target-oriented designed compounds as potential therapeutic agents against diseases produced by these parasites.

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 Full 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. Since 2006, she has dedicated her scientific interest to the identification and characterization of molecular markers of resistance in the *Leishmania* sp. infection model.

Leishmaniasis: The Biology of a Parasite

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Emilia Díaz and Alicia Ponte-Sucre

Abstract

One of the main challenges of therapeutic tools for the treatment of parasitic diseases, including leishmaniasis, is the interwinned relationship between therapeutic failure and drug resistance. In fact, some field parasites might be naturally resistant to classical drugs and additionally, current therapies may induce drug resistance. In fact, treatment failure in leishmaniasis has multiple causes. Some are related to drugs, such as pharmacokinetic properties, toxicity, use of sub-optimal doses, or high cost of treatment. Parasite-related grounds include chemo-resistance and tolerance. Last but not least, the host plays a fundamental role in this situation since the patient's immune status and the risk of re-infection if living in an endemic region might also contribute to therapeutic failure. All these features are at least partially responsible for the disappointing persistence and re-emergence of leishmaniasis, as well as its death and disability-adjusted life year toll worldwide. A better understanding of the disease itself and of drug resistance, its molecular basis, its consequences, and the definition of possible paths for better treatments may help improve this depressing picture. In the present volume experts in the field cover current knowledge and future trends of these and many other aspects of drug resistance in *Leishmania*. This initial chapter offers a general introduction to the biology of the parasite, a piece of information fundamental for the topics included in the book and the comprehension of challenges we currently face for this disease.

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