Current Pharmacology Reports (2020) 6:25–27
https://doi.org/10.1007/s40495-020-00209-6

COMMENTARY

Survival Strategies of *Leishmania* Parasite: Too Many Questions and Few Answers

Awanish Kumar[1]

Published online: 22 January 2020

Introduction

Leishmania is a pathogenic trypanosomatid protozoan parasite that causes a complex of diseases, called leishmaniases (cutaneous (CL), diffuse (DCL), mucocutaneous (MCL), and visceral (VL) leishmaniasis) [1]. These 4 major clinical forms of leishmaniases are particularly very diverse and VL is fatal in the absence of treatment among them. The transmission vectors (sandflies) of leishmaniasis were reported at the beginning of the twentieth century and even in the twenty-first century; the leishmaniases remain a severe public health problem. The leishmaniases cause considerable morbidity and mortality in man and the burden of this disease is also found across the globe [2]. There is a gradual increase in the risk factors for leishmaniasis worldwide. More than 10 million people worldwide are at risk due to the leishmaniases disease with at least 400 million people at high risk in endemic areas (http://www.who.int/tdr/diseases/leish/). Poverty, malnutrition, continuing widespread migration from rural to urban areas, and continuing fast urbanization worldwide play a major role in the increased susceptibility to leishmaniases. The standard treatment of leishmaniases involves the use of first-line drug pentavalent antimonial (SbV) compounds. In recent years, increasing numbers of clinical failures of treatment with established antileishmanial drugs (pentavalent antimonials, amphotericin B formulations, miltefosine) have been reported due to the development of *Leishmania* parasite resistance to these compounds [2]. Resistance to these drugs has been reported in several parts of the world; therefore, it is an urgent need to develop some new drug molecules for leishmaniases.

✉ Awanish Kumar
drawanishkr@gmail.com; awanik.bt@nitrr.ac.in

[1] Department of Biotechnology, National Institute of Technology, Raipur, Chhattisgarh 492010, India

Survival Mechanism of *Leishmania* Parasite Inside the Macrophage

Leishmania is a flagellated protozoan parasite that survives and multiplies within mammalian macrophages in amastigote form. *Leishmania* amastigote is an obligate intracellular form of the parasite that resides in the membrane-bound compartments of macrophages known as parasitophorous vacuole (PV). PV has an acidic environment, pH ranging from 4.7 to 5.2, and shares features with late endosomes/lysosomes. Available evidence suggests that PVs are considered a potent compartment for antigen processing in the *Leishmania* parasite [3]. Thus, inside PV, the unfolding of parasite proteins takes place following exposure to very low pH. Within the macrophages, *Leishmania* has a high menace of encountering those molecules that contribute to antigen recognition by T cells ($CD4^+$ or $CD8^+$), i.e., molecules of MHC class II and class I, respectively. Moreover, after macrophage activation, they are also vulnerable to being killed by antileishmanial molecules produced by host cells such as reactive oxygen intermediates (ROI) or reactive nitrogen intermediates (RNI). Several leishmanicidal molecules of the host such as MHC class II molecules, ROI, and RNI have been shown to access the lumen of PV or membrane [4], regardless if *Leishmania* survives in such a highly adverse condition. So, it arises too many questions regarding the survival mechanism of the *Leishmania* parasite inside the macrophage. (A) Do PVs provide shelter or a safe way for *Leishmania* survival? (B) Do PVs protect *Leishmania* from lysis of cytoplasmic content of macrophage? (C) What are the molecular mechanisms that recruit the construction of the large PV sheltering the pathogen *Leishmania*? (D) Do PVs provide a good niche from which *Leishmania* can readily withdraw nutrition? (E) Is the PV an appropriate place for the development of the complexes between MHC class II molecules and peptides of the *Leishmania* parasite? (F) What is the nature of the interactions intricate in the attachment of amastigotes to the membrane of PVs? These questions are unanswered until now. These questions are

needed to be answered. The researcher anticipates that there may be several possible strategies by which *Leishmania* might survive inside the PV and establish disease in the host, (a) by producing some molecules that inactivate or destroy the toxic ROS of macrophage components (ROI or RNI), (b) by infecting those macrophage populations that are impotent to synthesize leishmanicidal molecules, and (c) by infecting those macrophages whose activation is hampered (up to certain extent), or that have been disabled by contact with certain cytokines such as interleukin-4 (IL-4), IL-10, and tumor growth factor-beta (TGF-β).

The host macrophage's inability to convert leishmanicidal could result from numerous parasite-induced mechanisms. For example, the loss of co-stimulatory molecule expression of macrophages infected with *Leishmania* has been described. These molecules are usually vital to accomplish competent antigen-specific T cell activation. In addition, reduced MHC class II–restricted presentation of both parasite and non-parasite antigens by macrophages infected with *Leishmania* has been frequently described. Mechanisms functioning in the PVs could be the cause of this shortage. For example, the conditions of PV could be too unadorned for effective processing of most antigens, but intracellular handling of MHC class II molecules or the formation of peptide class II molecule complexes could also be modified by the presence of *Leishmania* parasites. It should be made vibrant that *Leishmania* belongs to a highly heterogeneous group of pathogens, and it is, therefore, possible that the PVs (where each species lives) have their individual inimitable characteristics, that are not much explored yet.

Mechanism and Emergence of Drug Resistance in *Leishmania* Parasite: a Wide Bore Fishing Net, More Holes than Threads

Extensive research has been done to elucidate the mechanism of antileishmanial resistance; however, the exact mechanism is not very much clear [5]. Few points are summarized below regarding our understanding of the mechanism of drug resistance in *Leishmania*. (a) There is a decrease of drug uptake inside the cell due to the loss of a transporter required for uptake. This drug uptake decrease contributes to resistance to various antileishmanial drugs. (b) The efflux of drugs from the *Leishmania* cell by P-glycoproteins and other traffic ATPases could possibly be an important mechanism of drug resistance. (c) Previous studies indicated that transmembrane transporters are involved in resistance to drugs in *Leishmania* spp. (d) PgpA P-glycoprotein of *Leishmania* spp. transports glutathione conjugates of arsenite and antimonials rather than the compounds themselves. (e) The altered expression of other transporters like aquaglyceroporins, ABC transporter MRPA, transporters for pentamidine uptake, and P-type transporter (LdMT gene) from the aminophospholipid translocase subfamily has been also reported in drug-resistant *Leishmania* parasites. (f) Loss of antileishmanial drug activation is the main mechanism of resistance. (g) Resistance of drug in *Leishmania* parasite also arisen through alteration of drug targets.

As we know, *Leishmania* parasites are accountable for some of the most devastating and prevalent disease (named leishmaniasis) of human and domestic animals [6]. Leishmaniasis is the collective name for several diseases caused by a flagellate protozoan parasite of the genus *Leishmania*, which has diverse clinical manifestations. Worldwide, over 17 species and subspecies of *Leishmania* infect humans, each of them causing a different spectrum of indications ranging from simple self-healing skin ulcers, to severe skin ulcers, to fatal/life-threatening VL. Drugs (therapeutic) and vaccine (prophylactic) are two major weapons against this disease. The treatment of leishmaniasis relies primarily on chemotherapy. The drugs available for treating *Leishmania* infections are very limited. Pentavalent antimonials (SbV), amphotericin B, pentamidine, and miltefosine are some marketed drugs used as antileishmanial therapy [7]. To interfere with parasite multiplication, a drug must reach its target cell where parasites reside. After activation inside the host cell, drug must damage the membrane of parasites. When drugs hit the target parasite, it must be sufficiently incapacitating to be killed. Each of these steps provides parasites with opportunities to interfere with the action of drugs ensuing drug resistance. After long use of SSG, the *Leishmania* parasite gets resistant to this drug. Mechanism behind drug resistance still remains unclear and the following question may arise regarding emergence of drug resistance against *Leishmania* parasite: (a) interaction of drug with target protein of *Leishmania* may be made less effective by increasing the level of competing for substrate; (b) inside the parasite drug may be inactivated, excreted, or modified; (c) drug uptake may not be possible due to loss of uptake system or alternation of *Leishmania* membrane components; (d) *Leishmania* parasite may evade drug action by hiding itself. Most resistance mechanisms known to date are related to a decreased uptake or increased efflux of the drug due to mutations or altered expression of membrane transporters. More in-depth knowledge is required in this direction and field.

Discussion

Our knowledge of the survival strategy of *Leishmania* inside the host and the emergence of drug resistance is not in-depth. As the armamentarium of antileishmanial is limited, it is important to prevent the spread of resistance. A better understanding of these points is necessary to unravel the puzzle of leishmaniasis. Survival of *Leishmania* parasite in harsh

conditions and resistance against various antileishmanial arise many questions but the satisfactory answer is unavailable. If we get the answer to these enigmas, it would be easy to make some more new rational drugs against leishmaniases to address the problem of drug resistance. Treatment outcomes with monotherapy have been consistently reported poor in the case of leishmaniases. Therefore, available antileishmanial and new rational drugs could be used in combination therapy for a more effective treatment for drug-resistant *Leishmania* parasite. Nanoparticles and nanobody (nanoparticles coupled to the fragment of single-domain heavy-chain antibody that specifically recognizes the parasite surface) could be also used for targeted drug delivery to overcome drug resistance. Development of nanocarrier design like nitric oxide-releasing nanoparticles, pH-sensitive formulations, and prodrug dendrimers could also improve the drug efficacy and mitigate the drug resistance problem in leishmaniases.

Acknowledgments The authors are grateful to the Dept. of Biotechnology, National Institute of Technology Raipur (Chhattisgarh), India, for providing space, facility, and resources for this work.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Ponte-Sucre A, Gamarro F, Dujardin JC, Barrett MP, López-Vélez R, García-Hernández R, et al. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017;11(12):e0006052.
2. Hefnawy A, Berg M, Dujardin JC, De Muylder G. Exploiting knowledge on Leishmania drug resistance to support the quest for new drugs. Trends Parasitol. 2017;33(3):162–74.
3. McConville MJ, Naderer T. Metabolic pathways required for the intracellular survival of Leishmania. Annu Rev Microbiol. 2011;65:543–61.
4. Faleiro RJ, Kumar R, Hafner LM, Engwerda CR. Immune regulation during chronic visceral leishmaniasis. PLoS Negl Trop Dis. 2014;8(7):e2914.
5. Chakravarty J, Sundar S. Drug resistance in leishmaniasis. J Glob Infect Dis. 2010;2(2):167–76.
6. Kumar A. Transmission of leishmaniasis from human to other vertebrates: a rapid zooanthroponotic evolution. Int Microbiol. 2019;22(3):399–401.
7. Kumar A, Pandey SC, Samant M. Slow pace of antileishmanial drug development. Parasitol Open. 2018;4(e4):1–11.