J Parasit Dis (Jan-Mar 2019) 43(1):1–13
https://doi.org/10.1007/s12639-018-1061-4

REVIEW ARTICLE

Dependence of *Leishmania* parasite on host derived ATP: an overview of extracellular nucleotide metabolism in parasite

Kashika Arora[1,2] · **Ambak Kumar Rai**[1]

Received: 30 June 2018 / Accepted: 24 November 2018 / Published online: 1 December 2018
© Indian Society for Parasitology 2018

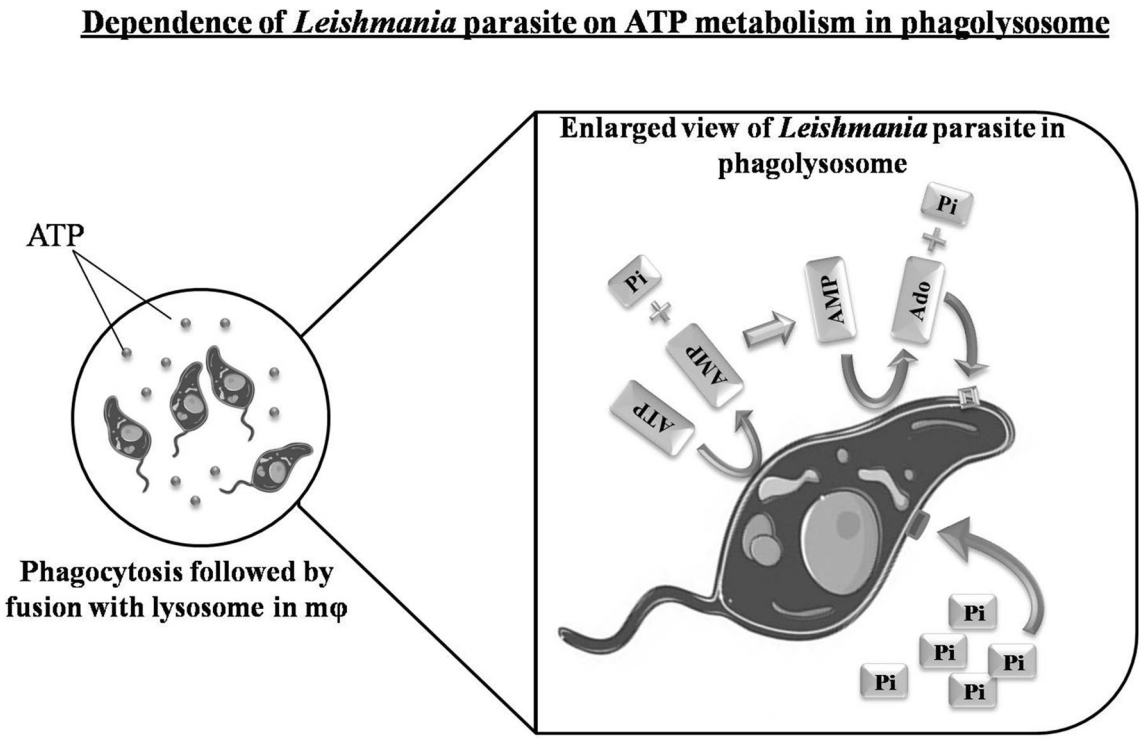
Abstract The widespread role of ATP in humans has been characterized as a substrate for metabolism as well as other key processes occurring in the human system. Hence, the ATP pool may also be accessible to the invaders, including *Leishmania donovani* (a protozoan parasite), considering the fact that ATP is not scarce at the cellular location where the pathogen resides. The protozoan parasite survives in the human host by utilizing purines from its extracellular environment, due to its inability to synthesize purines de novo. The purines are accessible in the form of nucleoside triphosphates (NTPs), for example, adenosine (ADO) in the form of ATP molecules. These NTPs are processed by the ecto-nucleotidases in the transmembrane region of the parasite, which are then transported inside via nucleoside/nucleobase transporters belonging to the ENT family of transporters. Besides, the breakdown of NTPs by ecto-nucleotidases also yields inorganic phosphate (Pi) as by-product which is utilized by the parasite to maintain Pi homeostasis. These transporters have been characterized in protozoan parasites exhibiting homology in various species of *Leishmania*. Once inside the parasite, these purines (or their derivatives) are fluxed into purine metabolic pathways with the help of several cytosolic enzymes, prominently, adenosine deaminase, adenine amino hydrolase, phosphoribosyl transferases (APRT, HGPRT and XPRT) and adenosine kinase. This review outlines the predominant role of extracellular nucleotide metabolism and intracellular metabolic machinery in the containment of leishmanial infection. It also highlights the importance of inorganic phosphate transporter in relation to the purine transport.

✉ Ambak Kumar Rai
ambakrai@mnnit.ac.in

[1] Department of Biotechnology, Motilal Nehru National Institute of Technology (MNNIT) Allahabad, Allahabad 211004, U.P., India

[2] Present Address: Biomedical Research Center, Ghent University Global Campus, Incheon 21985, South Korea

Graphical abstract



Keywords ATP · Nucleoside/nucleobase transporters · 5′-Nucleotidase · 3′-Nucleotidase · ADA · HGPRT · XPRT · APRT · Pi transporter

Abbreviations

NTP	Nucleoside triphosphate
ADO	Adenosine
GUO	Guanosine
INO	Inosine
PYR	Pyrimidine
Pi	Inorganic phosphate
LdNT	*Leishmania donovani* nucleoside transporter
Pu	Purine
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
XPRT	Xanthine phosphoribosyl transferase
APRT	Adenine phosphoribosyl transferase
AK	Adenosine kinase
AAH	Adenine aminohydrolase

Introduction

Leishmaniasis (Kala azar) is an infectious disease caused by protozoan flagellate parasite, i.e. *Leishmania*, transmitted by the bite of female *Phlebotomine* sand flies, categorised into cutaneous (CL), muco-cutaneous (MCL) and visceral leishmaniasis (VL) depending upon their pathological manifestations. The disease is endemic in 88 countries across the world with most impact on developing countries like India, Nepal and Bangladesh (World Health Organization 2010; Alvar et al. 2012; Ready 2014). According to World Health Organization report in 2014, more than 90% of new cases occurred in Brazil, Ethiopia, Somalia and Sudan, including India. VL is the most deadly form of the disease caused by *Leishmania donovani,* if remained untreated (Stauch et al. 2011). The parasite exists as flagellate promastigote in the sandfly and non-flagellate amastigote in humans. The experimental parameters influencing the pathogenecity in mice models have also been discussed recently (Loeuillet et al. 2016). However, the experimental form of the disease may not be the true reflection of its human form. Not only morphological, these two stages of the parasite are functionally very distinct (Dayakar et al. 2012). With the bite of the sand fly, these parasites along with the saliva enter inside the human host and disseminate to various visceral organs of the body, thus, complying with the term ‘Visceral Leishmaniasis’.

The problem in treatment of the VL patients has been aggravated by emergence of unresponsiveness to the first line drug i.e. sodium antimony gluconate (SAG). This has been further compounded by the side effects associated with the widely used drug Amphotericin B and its variants

(Ashutosh and Goyal 2007; Chawla and Madhubala 2010; García-Hernández et al. 2012; Leprohon et al. 2015). Recent reporting of unresponsiveness to Amphotericin B has raised the concern. Furthermore, the unresponsiveness to existing drugs (Singh et al. 2012; Rajasekaran and Chen 2015), side effects associated with new drugs (Gupta 2011), occurrence of post kala azar dermal leishmaniasis (PKDL), persistence of parasite reservoir etc. are major challenges and pose a threat of outbreak in coming years. It is believed among the scientific community that the disease has been incidentally contained by the National Malaria Control Programme sponsored by Government of India in the region. The anti-mosquito spray also restricts the sand fly colony and thus the spread of the disease. It is worth to mention the Kala-azar Eradication Programme of Government of India, which has been successful and gradually moving ahead toward the eradication of the disease. Despite of all these efforts, the disease can still be found in certain regional pockets of Bihar and adjoining states of India, which is a major threat to eradication of the disease. All these are indicative of the need to have a newer and better anti-leishmanial drug for disease management.

The parasitic behaviour is defined by the ability of any organism to seek host support for its survival. *Leishmania* parasite exhibits parasitism by extracting key substances from the host cells i.e. macrophages, for their maintenance and survival (Rodrigues et al. 2016). Unlike their mammalian host, *Leishmania* lacks the machinery for nucleotide synthesis. Acquisition of nucleotides from the host cells is one of the pathways crucial for the growth of *Leishmania* species. Being a unicellular eukaryote, the *Leishmania* parasite requires metabolic activities to generate energy for survival. Interestingly, these parasites do not have the ability to synthesize nucleotide rings de novo, and therefore, depends upon the salvage of preformed nucleotides from the host (Ullman et al. 2008; Boitz et al. 2012b; Boitz and Ullman 2013). The extracellular environment of the parasite comprises of purines, pyrimidines and their derivatives. For example, ATP, ADP, AMP, adenosine and adenine are available in the milieu possibly in the varying proportions. To facilitate their acquisition, parasite expresses various enzymes and transporters on their surface (Martin et al. 2014). These purine derivatives are generated by the catalytic activity of membrane-bound enzymes, known as ecto-nucleotidases (ecto-NTDases), which can then be transported inside the parasite via nucleoside/nucleobase transporters (Ortiz et al. 2007, 2009; Carter et al. 2010). The ecto-NTDases release pyrophosphates (PPi) from the nucleotides which can further be converted into inorganic phosphate (Pi) by pyrophosphatase (Cosentino-Gomes and Meyer-Fernandes 2011; Leite et al. 2012; Freitas-Mesquita and Meyer-Fernandes 2014). This reaction plays an important role in maintenance of Pi homeostasis in parasite, and thus, its survival per se.

Understanding the purine salvage pathway in detail would enable us to derive approaches for inhibition of either catabolism and/or transportation of purine across the membrane. Thus, it may possibly restrict the growth of the parasite. Based on various studies, it is observed that the purine salvage pathway is not a '*one-line*' pathway. Major steps of the pathway, if not all, are found to be bypassed and can be corroborated by another enzyme(s)/sub-pathway (Berg et al. 2010). Considering such proposition, perhaps not one drug, but possibly a combination of inhibitors may inhibit the growth of the parasite. In contrast to pyrimidine salvage pathway, distinct differences are reported in purine salvage pathway of the parasite and its mammalian counterpart (Van Den Berghe et al. 2012). However, it is essentially required to explore the exclusivity of the enzyme(s) or transporter(s) or their combinations for survival of parasite.

In this review, we have focused on the significance of purine (adenine) salvage pathway for the survival of *Leishmania donovani* parasite. Additionally, its role in visceralization of the Kala-azar disease and the possible involvement of an enzyme and/or transporter to support the aforementioned pathway has been discussed. The status of the purine salvage pathway in drug-resistant and drug-sensitive parasites has also been analysed. Besides the critical examination of available literature relating to our approach, we have also highlighted the future potential methods for the containment of the parasite.

Nucleotide metabolism in *Leishmania* parasite and its dependence on host

Acquisition of ATP and/or its derivative may considerably be different between the two stages of the parasite, flagellate promastigote and aflagellate amastigote. In the disease process, the promastigote enters in the macrophages through phagosome, which then fuses with the lysosome and the environment within the phagolysosome tries to disrupt the promastigotes. In turn, promastigote converts into amastigotes with the phagosome/phagolysosome. This process in phagosome coincides with the routine process of removal of cell debris, immune complex, dead/decaying RBCs (if in spleen). The catabolic processing of such cellular waste may lead to release of nucleotides in the environment surrounding the parasite within the phagosome/phagolysosome. In human form of the disease, the parasite load varies among the visceral organs like spleen, liver and bone marrow. However, it is believed that the site-specific immune regulation in these organs is in general attributed to the participating immune cell populations interacting with the parasites. Intrigued by this observation, it seems relevant to study the level of nucleotide supply to

the parasite residing within the tissue-resident macrophages and its consequences on parasite load at a given site.

As indicated above, the nucleotides in the extracellular milieu are processed by the enzyme(s) expressed on the surface of the parasite. Similarly, its human counterpart also expresses ATP catabolic enzymes in phagosome/phagolysosome (Pinheiro et al. 2006). Considering the opposing effect of ATP and adenosine on immune response, the host prefers to neutralize the nucleotide. Conversely, it can be theoretically presumed that the competitive acquisition by parasite within the microenvironment of the phagolysosome favors its survival. As per our literature survey, there is no study comparing the activity of host ATPase to that of parasite in the phagolysosome along with their ability of competitive nucleotide acquisition, if any. ATP → ADP → AMP → ADO is the reaction catalyzed by nucleotidases. The plasma membrane of the protozoan parasite is equipped with surface membrane bound ecto-enzymes. These enzymes are described as 3′- and 5′-nucleotidase in *Leishmania* that show functional homology to CD39 and CD73 respectively, in humans. The cyclic flow diagram of ATP metabolism is shown in Fig. 1 and the following section describes the properties of the crucial enzymes involved in the leishmanial infection. Purine salvage and its interconversion in *Leishmania* is facilitated by a number of enzymes like phosphoribosyltransferases, nucleoside deaminases, nucleoside kinases, nucleoside hydrolases etc., to name a few. Out of these, the crucial enzymes for scavenging nucleobases or nucleosides are Hypoxanthine–guanine phosphoribosyl transferase (HGPRT), Xanthine phosphoribosyl transferase (XPRT), Adenine phosphoribosyl transferase (APRT) and Adenosine kinase (AK). Adenosine, being the key purine for the establishment of infection, can be salvaged into inosine or adenine by adenosine deaminase (ADA) and adenosine hydrolase respectively. The anti-inflammatory nature of adenosine as a result of an effective T_H2 response has been previously reported (Faleiro et al. 2014). Besides, the concentrations of adenosine are also proved to be high during the leishmanial infection (Rai et al. 2011). The adenosine can be re-converted to AMP by adenosine kinase (AK) and *L. donovani* parasites are known to express high levels of AK (Rai et al. 2011). This enzyme from *L. donovani* has been isolated and characterized previously (DATTA et al. 2005). It has been observed that the addition of adenosine increases the cell number of the parasite significantly in vitro and its deficiency leads to survival up to 2 weeks, however, the cell number of the parasite is reduced significantly (Rai et al. 2011). Earlier, it was demonstrated that the saliva of the sand fly contains adenosine and inoculation of parasite with sand fly's saliva is essential for establishment of the infection (Kato et al. 2007). Furthermore, intra-dermal challenge of animal with parasite does not lead to establishment of infection (Côrtes et al. 2010). However, challenging animals with parasite along with salivary gland extract of the sand fly not only establishes the infection but also resembles with the human form of infection (Andrade et al. 2007). This has further been strongly supported by the findings where addition of adenosine at the time of infection increases the lesion size and inhibition of adenosine receptor A2B decreases the lesion size (de Almeida Marques-da-Silva et al. 2008). Moreover, the parasites causing non-healing disease show increased nucleotide hydrolysis as compared to parasite of self-healing form of the disease (de Almeida Marques-da-Silva et al. 2008). The properties of these ecto-nucleotidases are mentioned in the next section.

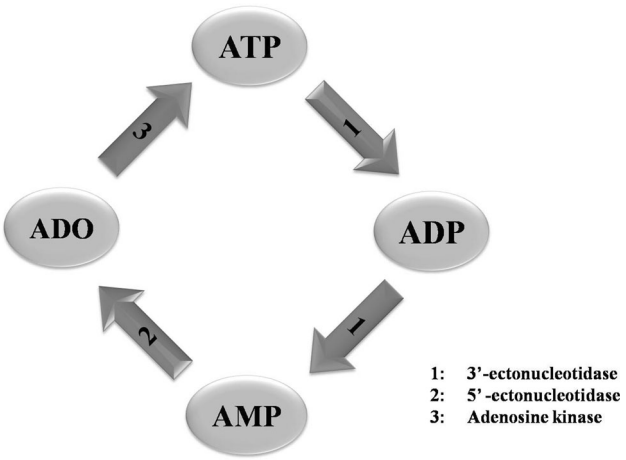


Fig. 1 Enzymes involved in catabolism of ATP located on the surface of *Leishmania donovani* parasite. CD39 and CD73 are the analogues of 3′-ecto-nucleotidase and 5′-ecto-nucleotidase, respectively, in humans. (ADO = Adenosine)

As per our literature survey, the interaction of drugs with the nucleotides in the extracellular milieu is not yet established. The antimony is abandoned from the clinical practices in Indian subcontinent after being the first choice drug for almost half a century (Ashutosh and Goyal 2007). Even then, the translocation of active drug components to the parasite and/or infected cells is not yet completely elucidated. Adenine is shown to make complex with the pentavalent form of antimony (Baiocco et al. 2009). Interesting to note that this complex formation occurs at acidic pH (pH 5), but not at neutral pH (pH 7.2–7.4), suggesting its role in translocation of the drug across the parasite membrane in the phagolysosome (Baiocco et al. 2009).

Enzymes required for extracellular catabolism of ATP

Ecto-NTDases are a class of enzymes in the transmembrane region that have their active site towards the extracellular surface (Robson et al. 2006). These enzymes are designated as 3′-nucleotidase and 5′-nucleotidase hydrolyzing 3′-nucleotides and 5′-nucleotides, respectively (Manzano and Castanys 2013). The localization of these enzymes in the surface membrane has been confirmed in *L. donovani* and clearly distinguished from another surface membrane enzyme known as non-specific acid phosphatase (de Almeida Marques-da-Silva et al. 2008; Guimarães-Costa et al. 2014). The sole purpose of these ecto-enzymes in the salvage of purines is to hydrolyze the nucleotides with the release of corresponding nucleosides and Pi. These enzymes are Mg^{2+}-dependent and known to vary between virulent and avirulent strains of *L. amazonensis* upon stimulation (Peres-Sampaio et al. 2008). Expression of ectonucleotidases is directly linked to the establishment of the infection and their progression (de Almeida Marques-da-Silva et al. 2008). Promastigotes of *L. amazonensis,* if cultured for long duration, induce smaller lesion and shows poor infectivity. In contrast, promastigotes cultured for short duration exhibit increased infectivity and produce larger lesion. This increased infectivity was proved to be associated with a high expression of ecto-nucleotidases (de Souza et al. 2010). On a similar note, the virulent parasites have been reported to hydrolyze more ATP and express more nucleotidases. Furthermore, the expression of nucleotidases is directly linked to the lesion size upon infection with parasite. Moreover, the animal challenged with long term cultured parasites showed enhanced immune response (IFN-γ ↑) and smaller lesions (de Souza et al. 2010). It is indicative of the fact that these ectonucleotidases are potentially a critical factor for virulence of the parasite. Besides, it may be concluded that the expression of ectonucleotidases on the surface of parasite is inversely correlated with the immune response mounted by the host. In the following section, we will be discussing the prominent nucelotidases in detail.

Ecto-ATPases

Although, the virulent role of ectonucleotidases has been elaborated in the previous sections, there are other ecto-enzymes on the surface of the parasite that act as ecto-phosphatases. Ecto-ATPases are a class of enzymes that primarily hydrolyze triphosphates. Like the aforementioned ecto-nucleotidases, these are divalent cation-dependent enzymes required in millimolar concentrations by the parasite (Almeida-Amaral et al. 2006). They are unique in a manner that they do not require high specificity for their action (Cosentino-Gomes and Meyer-Fernandes 2011). These ecto-ATPases basically belong to E-class of ecto-ATPases (Cosentino-Gomes and Meyer-Fernandes 2011) that also includes ATP diphosphohydrolases discussed below. The optimum pH for the activity of ecto-ATPases lies in the alkaline range. However, their expression is observed more in case of *L. amazonensis* amastigotes than promastigotes (Giarola et al. 2014). The activity of ecto-ATPases has been shown to be influenced by heat shock as well (Peres-Sampaio et al. 2008). These enzymes tend to show more ATPase activity when exposed to sudden increase in temperature. These properties of ecto-ATPase are suggestive of parasite virulence and survival. A recent research indicated a proportional increase in ecto-ATPase activity and drug resistance in case of *L. amazonensis* (Giarola et al. 2014). Also, the Mg^{2+}-dependent ecto-ATPase activity has been shown to be more effective in case of infective form of protozoan parasite as compared to non-infective form (Saad-nehme et al. 2004).

Ectonucleotide diphosphohydrolase

The E-class of ecto-ATPases also includes diphosphohydrolases which, as the name indicates, hydrolyze both nucleotide tri- as well as di-phosphates. This enzyme, otherwise, exhibits the same chemical properties (alkaline pH range and requirement of divalent cations for activity) as ecto-ATPases (Fonseca et al. 2006) and is located in the plasma membrane of any pathogenic protozoan with its active site towards the extracellular environment (Pinheiro et al. 2006). However, golgi-located NTPDase has been shown to be virulent at insect stage whereas the secretory NTPDase can be dispensed, thus playing no role in virulence (Sansom et al. 2014). The enzyme has been characterized in many of the protozoan parasites and shows the same characteristic properties. Although there have been many inhibitors reported for the ecto-ATPase class of enzymes (de Souza Leite et al. 2007; Weiss et al. 2015), but these inhibitors do not show any promising inhibition of survival rate of parasites in the host. In addition, the activity of diphosphohydrolases is not affected by other ATPase and phosphatase inhibitors (Pereira-Neves et al. 2014). This property of diphosphohydrolase is suggestive of its uniqueness among other ATP-hydrolyzing enzymes. The ecto-NTP diphophohydrolases exhibit homology to CD39 in humans and have several isoforms (NTPDase1-8) reported in protozoans (Zimmermann 2001). The inhibitors of NTPDases have been shown to alter the purinergic receptor activation (P1 and P2) in humans (Brunschweiger et al. 2008) with an exception of a commercially available ATP analogue ARL-67156 which is a broad spectrum drug

(Robson et al. 2006). Hence, it is difficult to target these ecto-enzymes for drug therapy.

3′-Nucleotidase/nuclease

3′-nucleotidase (EC 3.1.3.6) is a surface membrane-bound enzyme reported in case of *Leishmania* species. It has a molecular mass of 43 kDa with pH optimum in the range of 5.5-7.5 (Guimarães-Costa et al. 2014). This enzyme belongs to class I nuclease family hydrolyzing single-stranded DNA & RNA exhibiting homology to S1 & P1 nucleases of *Aspergillus* and *Penicillium* (Maxwell et al. 2008). Various researchers report that this enzyme is not present in mammalian cells despite the presence of 3′-nucleotides in tissues like spleen (Paes-Vieira et al. 2018); however, CD39/ATP diphosphohydrolase plays a similar role in humans (Dwyer et al. 2007). 3′-nucleotidase acts upon ribonucleoside 3′-phosphates, preferably 3′-AMP and 3′-IMP, as observed in *Leishmania mexicana mexicana* (Cummings et al. 2012). A contrasting, yet similar effect of this enzyme has been observed in *L. amazonensis* where it acts on 3′-AMP and yields adenosine which is otherwise catalyzed by 5′-nucleotidase (Paletta-Silva et al. 2011). The functional domains of this enzyme have been identified in *L. donovani* characterized as a cytosolic N-terminal signal peptide, a C-terminal transmembrane domain and an N-linked glycosylation site (Naderer et al. 2004). Out of all the functional domains, the signal peptide was reported to make a difference with reference to the survival of the parasite. This was noticed when this peptide was made deficient resulting in sole expression of the enzyme in cytoplasm which was toxic to the parasite. So, the modification in the functional domains of 3′-nucleotidase may help in creating toxic environment for *Leishmania* (Smith et al. 2007).

5′-Nucleotidase (AMP → Adenosine)

Besides 3′-nucleotidase, another ecto-NTDase viz. 5′-nucleotidase (EC 3.1.3.5) performs the hydrolysis of nucleoside monophosphates to their respective nucleosides (Hunsucker et al. 2005). This enzyme is classified on the basis of localization in glycolipid-rich membrane, in mitochondria and in cytosol in humans whereas *Leishmania* parasite consists of only the surface membrane bound form of 5′-ecto-nucleotidase (Składanowski 2013). A total of 7 forms of 5′-NTDase have been reported till now in mammals, out of which only 5′-ecto-NTDase lies in the transmembrane domain homologous to leishmanial enzyme. However, CD73 in humans has shown to be homologous to this enzyme. This enzyme is stabilized by glycophosphatidylinositol (GPI) anchor and has a much higher affinity for its substrates than intracellular 5′-nucleotidases (Składanowski 2013). Out of all the nucleoside monophosphates, AMP is the most preferred substrate which is converted to adenosine by its action. The localization of the ecto-NTDases has been confirmed and characterized in parasites (Paes-Vieira et al. 2018). There may be a risk in targeting this ecto-NTDase as it may affect the host due to sequence homology. But the functional domains of the enzyme should be analyzed to get a clear picture of its action.

Adenosine deaminase (ADA) and kinase (AK)

As soon as the nucleoside is sensed in the enviroment, its fate may be decided by enzymes like Adenosine deaminase (ADA) (EC 3.5.4.4) and adenosine kinase (AK). ADA catalyzes the conversion of adenosine to inosine which can be fluxed to purine metabolic pathway. The ADA enzyme in the human host, extracellular to the parasite, will perform the same function. The inactivity of this enzyme increases deoxyadenosine (Kato et al. 2007). On the other hand, kinases phosphorylate the adenosine and these enzymes are localized in the cytosol of the parasite exclusively (Yegutkin 2008; Boitz et al. 2012a). However, high concentrations of adenosine have been reported even in the presence of high ADA levels (Rai et al. 2011). Although, these enzymes are crucial to the parasite, the survival rate is not much affected by their deficiency.

Influence of Pi homeostasis in parasite survival

The released Pi by the action of ecto-NTDases on nucleotides is utilized by the parasite for Pi homeostasis as an alternate mechanism of survival. For instance, the decrease in survival rate has been observed by disturbing the concentrations of Pi in *L. infantum* (Vieira et al. 2011). Further, it can be postulated that there exists a Pi transporter in the plasma membrane of the parasite which is ultimately allowing the uptake of Pi from the extracellular environment of the parasite (Dick et al. 2014). It has been shown that decrease in Pi concentration can upregulate the expression of Pi transporters. Also, concentration of Pi may influence the activity of 3′-NTDase, as proved in case of *Leishmania infantum* (Vieira et al. 2011), or the presence of ATP may affect the uptake of Pi. This increase in activity is thought to be an adaptive mechanism to survive under stress conditions by providing purines and Pi (Paletta-Silva et al. 2012). Any reports suggestive of affecting the survival of *Leishmania* by inhibiting the Pi transporter can be considered as an alternate mechanism for disrupting the survival of *Leishmania* parasites.

Extracellular nucleoside/nucleobase transporter

Once the nucleosides are formed by the action of ecto-NTDases, the next step is to transport these nucleosides/nucleobases across the plasma membrane of the parasite. The nucleoside and nucleobase transporters have been reported in parasitic protozoa wherein each transporter is specific (Landfear et al. 2004; Young et al. 2008). There are two families of these transporters viz. Equilibrative Nucleoside Transporters (ENTs) and Concentrative Nucleoside Transporters (CNTs) working as permeases (Young et al. 2008). The ENTs differ from CNTs in the context that they do not concentrate their substrates but simply mediate flux down a thermodynamic gradient (Landfear 2008). However, ENT family members from *L. donovani* are reported as electrogenic proton symporters (Stein et al. 2003), but this does not confirm their role as concentrative transporters. The members of the CNT family are sodium symporters found predominantly in humans (Landfear et al. 2004) whereas ENTs are identified in both mammals and protozoans (Young et al. 2008). The ENTs act as the driving force for the uptake of nucleosides into the *L. donovani* parasite by generating a proton electrochemical gradient across its plasma membrane (Ortiz et al. 2009). Surprisingly, the utilization of this proton gradient results in the concentrative uptake of nucleosides, thereby behaving as CNTs despite of no similarities at the sequence level (Stein et al. 2003). Clearly, this demonstrates the dependence of parasite on ENTs for the purine uptake.

The transport of ATP in *Leishmania* species is governed by ABC (ATP-Binding Cassette) transporters, as in the case of humans (Manzano and Castanys 2013). These transporters, along with the transport of certain compounds, are responsible for drug resistance in the parasite (Sauvage et al. 2009). Resistance to antimony by *L. major* has been reported due to the presence of an ABC half-transporter designated as ABCI4 (Manzano and Castanys 2013). However, miltefosine has proved to be an effective oral drug against VL, even in antimony-resistant cases as well (Saad-nehme et al. 2004). Three different classes of leishmanial ABC transporters are responsible for translocation of compounds and presumably responsible for development of drug-resistant cases which show homology with mammalian ABC transporters (viz. Pgp1 & MRP1) (El Fadili et al. 2005). Also, considering the structure of ABC transporters, they consist of ATP-binding domain which is conserved among various species (Parodi-talice 2001). Their homologies with mammalian ABC transporters make it difficult to set them as a target for therapy against *Leishmania*. However, the nucleoside/nucleobase transporters identified can behave as potential targets.

Specific nucleotide/nucleobase transporters in *L. donovani*

Leishmania donovani expresses two high affinity transporters that belong to Equilibrative Nucleoside Transporters (ENTs) with non-overlapping substrate specificities (Sanchez et al. 2004). These are characterized as LdNT1 and LdNT2, wherein LdNT1 is specific for transport of adenosine and pyrimidines (Liu et al. 2006) and LdNT2 is specific for inosine and guanosine (Landfear et al. 2004). The roles of these permeases have been confirmed by targeted gene replacement technology (Carter et al. 2001). Similarly, in case of other strains of *Leishmania* parasite like *L. major*, four ENT permeases help in nucleoside/nucleobase transport. They are designated NT1 for adenosine and pyrimidine nucleosides (Carter et al. 2001); NT2 for inosine, guanosine and xanthosine (Carter et al. 2000); NT3 for purine nucleobases (Sanchez et al. 2004); and NT4 having low affinity for adenine (Ortiz et al. 2007). The nucleoside transporters belonging to *L. donovani* parasites exhibit approx. 33% identity with mammalian ENTs at transcriptional level (Young et al. 2008). Since, the function of both mammalian and parasitic ENTs is same; therefore, the identity between the two can conclude that the active site for transport is encoded by the region common between the two. However, the difference lies in the limited substrate specificity of parasitic transporters compared to hENTs which accept all nucleosides (Ortiz et al. 2007).

The genes encoding LdNT1 have been cloned and sequenced by functional rescue viz. LdNT1.1 and LdNT1.2 (Alzahrani et al. 2017). The point mutations in nucleoside transporter gene have proved that LdNT1.1 is expressed exclusively in promastigotes whereas LdNT1.2 in amastigote stage of the parasite (Vasudevan et al. 2001; Galazka et al. 2006). Both the genes have been shown to differ at six positions at transcription level (Galazka et al. 2006). Hence, it can be hypothesized that LdNT1.1 and LdNT1.2 are two alternate forms of a single gene expressed in different life cycle stages of the parasite.

Also, 33% identity has been reported in case of *L. major* NT3 (LmaNT3) with LdNT1.1 (Sanchez et al. 2004) that presumes the importance of LmaNT3 in the survival of the parasite. The null mutants for LmaNT1 and LmaNT2 have been generated together that resulted in viable parasites (Liu et al. 2006). The null mutants for LmaNT3 were also viable but were unable to sustain infection at promastigote stage as compared to wild-type or NT3-complemented mutants (Ortiz et al. 2007). Hence, impairment in adenosine transport is crucial in treating leishmanial infection.

Nucleobase transporters identified in *L. donovani* are LdNT3, which allows the transport of adenine, guanine and hypoxanthine, as well as LdNT4, which transports only the

nucleobase adenine (Ullman et al. 2008). LdNT4 is thought to have low affinity for adenine as compared to LdNT3 and is active at acidic pH only (Ortiz et al. 2009). Therefore, LdNT4 is expressed predominantly in case of amastigotes, whereas LdNT3 in case of promastigotes. Owing to the functionality of LdNT3 and LdNT4, they can be described as alternate forms of a single gene. However, there has been no report of low expression of LdNT3 in case of amastigotes. The antibiotics against specific transporters have been introduced (mentioned in forthcoming section) but their efficacy has not been tested in case of VL-causing parasite. The detailed study about the active sites of these transporters may result as a useful approach for the mankind.

Biochemistry of ENTs

The structures of the members of transporter families are essential to be determined for examining the transport of important substrates from host milieu to the cytosol of the parasite. Out of the transporters mentioned above, the structural characterization of ENTs is very significant. The human ENTs (hENT1 & hENT2) are nucleobase and nucleoside transporters that show 40% identity in amino acid sequence and consist of 11 transmembrane domains. This topology is shown to be exhibited by all members of the ENT family (Podgorska et al. 2005). The interaction of a high-affinity competitive inhibitor nitrobenzylmercaptopurine ribonucleoside (NBMPR) with hENT1 postulated that transmembrane domains 3–6 (TMD3–TMD6) bind to substrates (Cano-Soldado and Pastor-Anglada 2012). Substrate recognition, specifically in hENT1, is aided by G179 in the helix of TMD5 wherein site-specific mutagenesis impairs the transportation (Landfear et al. 2004). Similarly, in case of *Leishmania*, the LdNTs are composed of 11 transmembrane regions, out of which, TMD5, TMD7 and TMD8 play a significant role in substrate selectivity, particularly in LdNT1 and LdNT2 (Valde et al. 2004).

The signature residues that are thought to be responsible in the translocation pathway of adenosine are G-183 in TMD5 and C-337 in TMD7 (Vasudevan et al. 2001). It has been postulated that mutation of G-183 by any bulkier amino acid residue hampers the translocation pathway. However, replacement with alanine has no effect on the transport (Vasudevan et al. 2001). This accounts for the fact that both factors (low molecular weight & nature of the amino acid), play a crucial role in facilitating translocation of the substrates. With due consideration, it has been observed that presence of four consecutive glycine residues provide conformational flexibility for binding of the nucleoside in TMD5 (Eilers et al. 2000), wherein G-183 acts as the substrate-binding pocket. Interestingly, the comparison of these signature residues in LdNT1.1 and LdNT1.2 reveals no differences at the sequence level. So, it can be hypothesized that there must be some other factors that are causing these genes to be expressed during different life cycle stages of the parasite. Similarly, in case of LdNT2, which is an inosine-guanosine transporter, Asp-389 and Arg-393 are involved in translocation of substrates (Carter et al. 2000). The detailed information about the secondary structure of transporters will help in inhibiting the transportation across them.

Metabolic machinery in the parasite

Purine metabolism has been reported to be essential for the survival of *Leishmania* parasite and hence, promotes the salvage of purines from the extracellular environment. So, the influx of purines via nucleoside/nucleobase transporters, maintain the metabolic machinery of the parasite which marks the role of other metabolic enzymes involved known as phosphoribosyl transferases (PRTs). These enzymes of purine salvage utilize Phosphoribosyl Pyrophosphate (PRPP) as a substrate catalyzing the phosphoribosylation of purine bases (Mondal et al. 2014). A study on the purine salvage in *Leishmania* parasite has revealed the significance of each of the enzymes involved in nucleotide metabolism as well as salvage of purines (Boitz et al. 2012b). However, it has been concluded that out of the three phosphoribosyl transferases viz. Hypoxanthine–guanine phosphoribosyl transferase (HGPRT), Xanthine phosphoribosyl transferase (XPRT), Adenine phosphoribosyl transferase (APRT); only HGPRT and XPRT play a significant role as their respective null mutants ($hgprt^-$ & $xprt^-$) as well as double null mutants ($hgprt^-/xprt^-$) affect the viability of the parasite. On the contrary, null mutants of APRT ($aprt^-$) could effectively incorporate adenine into the nucleotides (Boitz and Ullman 2006). This is basically due to the availability of enzymes in parasite that convert all purines into the form catalyzed mainly by HGPRT and XPRT (Boitz et al. 2012b). Since, XPRT lacks in the mammalian genome and owing to the significance of this enzyme in parasite, it can be a potential target against the protozoan.

Other metabolic enzymes

There are other enzymes reported in protozoans, the deficiency of which will not affect their viability in a significant manner. However, the null mutants generated together for each one of them will surely provoke the parasite to search for an alternative mechanism of survival. One of these enzymes is characterized as Adenine aminohydrolase (AAH) which catalyzes the irreversible deamination of adenine to hypoxanthine (Boitz et al. 2012a). Although, there

is no counterpart of AAH in humans, but the *L. donovani* AAH shows 23% identity with human adenosine deaminase (ADA). The role of AAH was determined by generating null mutants of HGPRT and XPRT, and blocking AAH with 2′-deoxycoformycin (dCF), a potential inhibitor of AAH (Boitz et al. 2012a). AAH is active in both promastigote and amastigote life cycle stage of *Leishmania*. The mammalian ADA is also inhibited by dCF (Yegutkin 2008) implying that the similarity region between mammalian ADA and parasitic AAH includes the active site for these enzymes. Therefore, the active site cannot be utilized as a source of treatment in humans. However, other significant regions must be explored using bioinformatics approach for designing an AAH inhibitor combined with XPRT as the target. The transporters for the salvage of enzymes present in human counterparts are not generally required by the parasite as they synthesize their own enzymes.

Relevance of extracellular ATP catabolic enzyme(s) and/or its transporter for the containment of Leishmaniasis

ATP is provided to the *Leishmania* parasite in the extracellular form by the human host. The catabolic enzymes, predominantly ecto-nucleotidases, in the transmembrane region of the parasite cleave the nucleotides and make them available to the surface transporters for importing adenosine, and other nucleobases, across the plasma membrane. As mentioned previously, adenosine is the prominent cause of leishmanial infection in humans. Hence, these catabolic enzymes as well as the nucleoside and nucleobase transporters, particularly of purines and their derivatives, help the parasite in sustainability and containment of infection in the human host. The overview of the transporters and enzymes involved in the acquisition of purines in the *Leishmania* parasite is depicted in Fig. 2.

Potential experimental drugs for the treatment of VL

Although various drugs have been introduced for the immune exclusion of the *L. donovani*, there are thrust areas that need to be considered for designing any new drug due to the resistance developed in the parasite. This resistance is thought to be induced by the presence of an intracellular ABC-half transporter reported in *L. major* that has shown to be resistant mainly against antimonial drugs, as mentioned before (Manzano and Castanys 2013).

Attempts are being made to design drugs with nucleobase and nucleoside transporters as drug targets. Some of

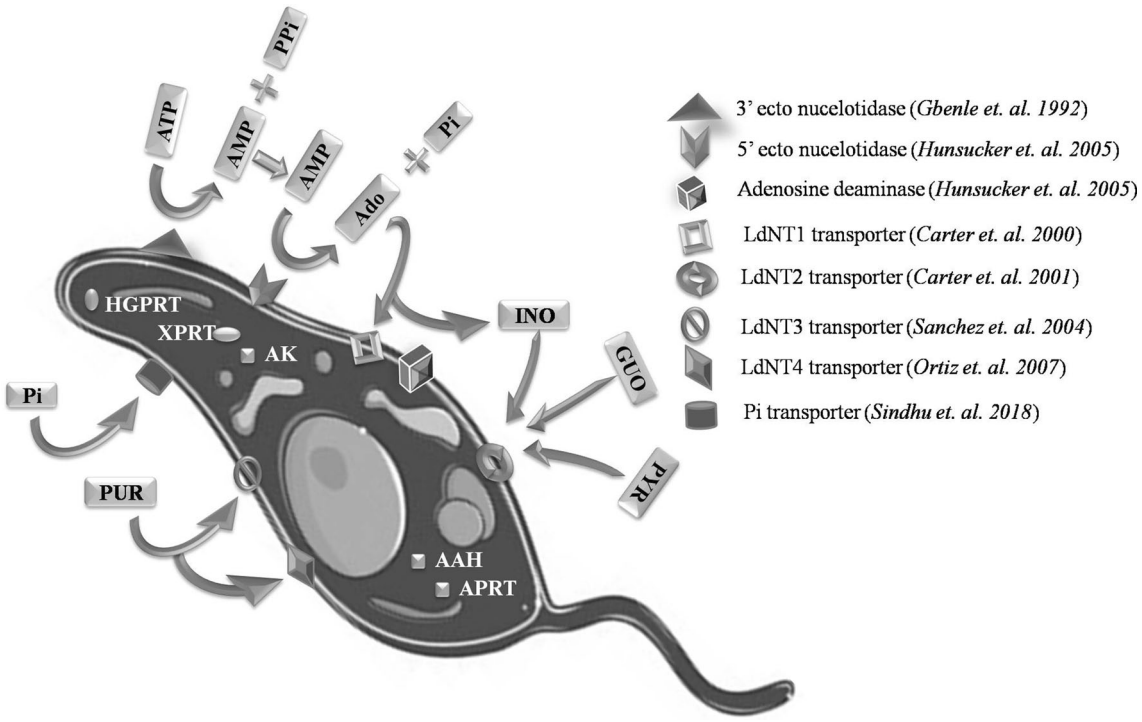


Fig. 2 Overview of ecto-NTDases and transporters involved in purine salvage pathway of *Leishmania* parasite along with key purine metabolic enzymes. *ADO* adenosine; *GUO* guanosine; *INO* inosine; *PYR* pyrimidine; *Pi* inorganic phosphate; *LdNT Leishmania donovani* nucleoside transporter; *Pu* purine; *HGPRT* hypoxanthine–guanine phosphoribosyl transferase; *XPRT* xanthine phosphoribosyl transferase; *APRT* adenine phosphoribosyl transferase; *AK* adenosine kinase; *AAH* adenine aminohydrolase

Table 1 Table shows the amino acid residues, their respective positions and location in transmembrane region differing in LdNT1.1 & LdNT1.2 transporters in *Leishmania* parasite

Position of a.a. residue	Amino acid in LdNT1.1	Amino acid in LdNT1.2	Transmembrane domain (TMD)	References
43	Proline	Serine	TMD1	Ready (2014), Rodrigues et al. (2016)
107	Methionine	Isoleucine	b/w TMD2 & TMD3	
160	Threonine	Alanine	b/w TMD4 & TMD5	
489	Alanine	Glutamate	Terminal residue	
490	Threonine	Arginine	Terminal residue	
491	Tyrosine	Histidine	Terminal residue	

the experimental drugs currently used include tubercidin for LdNT1 (Fukuda and Schuetz 2012), formycin B for LdNT2 (Carter et al. 2000) and allopurinol for LmaNT3 (Al-Salabi and De Koning 2005). The resistant cell lines TUBA5 and FBD5 have been isolated by introducing transport-functional mutation in LdNT1 and LdNT2, respectively, of wild-type strain (DI700) (Galazka et al. 2006). These cell lines have helped in the characterization of LdNTs on the functional basis.

Conclusion

Leishmania donovani, the most prominent strain of *Leishmania*, has been proved to be fatal causing Visceral Leishmaniasis. The parasite lags behind human metabolic machinery due to the absence of de novo purine synthesis, and thus, has to be dependent upon its host for the same. The processing of purine nucleotides in the extracellular environment is catalyzed by a special class of enzymes known as ecto-nucleotidases. However, the activity of these enzymes is thought to be affected by inorganic phosphate concentration in the extracellular milieu of the parasite. The main reason behind this affect seems to be the maintenance of Pi homeostasis for cell survival. The uptake of Pi released is facilitated by Pi transporter, which has been recently identified in *Leishmania donovani* by our group (Sindhu et al. 2018).

The purine metabolism is well-characterised in the parasite, along with the enzymes involved in it. Many of the enzymes have their counterparts in humans that prevent them from being used as drug targets. While, there are some enzymes unique to the parasite, the absence of which is fulfilled by the alternative mechanism. This calls for targeting the pathway that allows the *salvage* of purines, which marks the importance of nucleoside and nucleobase transporters. The transporters for purines in *L. donovani* have been explored and identified as LdNT1-4, among which, some signature residues have been reported to be significant in the translocation of purines across the plasma membrane. The key differences in the amino acid residues of LdNT1.1 and LdNT1.2 are shown in Table 1. The experimental drugs targeting the functionality of these transporters include tubercidin, formycin B and allopurinol with the development of resistant cell lines viz. TUBA5 and FBD5. The residues in LdNT3 and LdNT4 still needs to be elaborated. LdNT1.2 and LdNT4 work at acidic pH, and therefore, are functional when parasite is surviving in the phagolysosome of the human host, whereas rest of the LdNTs can be found in both, amastigotes as well as promastigotes. To conclude, a combined therapy with the drugs against the active site of LdNT1-4 as well as Pi transporter can prove to be efficient in disturbing the metabolic machinery of the parasite.

Acknowledgements The study is undertaken as part of dissertation work of Ms. Kashika Arora for the fulfilment of her postgraduate degree. The authors thank Motilal Nehru National Institute of Technology (MNNIT) Allahabad for supporting the study.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Almeida-Amaral EED et al (2006) *Leishmania amazonensis*: characterization of an ecto-phosphatase activity. Exp Parasitol 114(4):334–340. https://doi.org/10.1016/j.exppara.2006.04.011

Al-Salabi MI, De Koning HP (2005) Purine nucleobase transport in amastigotes of *Leishmania mexicana*: involvement in allopurinol uptake. Antimicrob Agents Chemother. https://doi.org/10.1128/aac.49.9.3682-3689.2005

Alvar J et al (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE. https://doi.org/10.1371/journal.pone.0035671

Alzahrani KJH et al (2017) Functional and genetic evidence that nucleoside transport is highly conserved in Leishmania species: implications for pyrimidine-based chemotherapy. Int J Parasitol Drugs Drug Resist. https://doi.org/10.1016/j.ijpddr.2017.04.003

Andrade BB et al (2007) Role of sand fly saliva in human and experimental leishmaniasis: current insights. Scand J Immunol 66:122–127. https://doi.org/10.1111/j.1365-3083.2007.01964.x

Ashutosh SS, Goyal N (2007) Molecular mechanisms of antimony resistance in Leishmania. J Med Microbiol 56(PART 2):143–153. https://doi.org/10.1099/jmm.0.46841-0

Baiocco P et al (2009) Molecular basis of antimony treatment in Leishmaniasis. J Med Chem 52:2603–2612. https://doi.org/10.1021/jm900185q

Berg M et al (2010) Inhibitors of the purine salvage pathway: a valuable approach for antiprotozoal chemotherapy? Curr Med Chem. https://doi.org/10.2174/092986710791556023

Boitz JM, Ullman B (2006) A conditional mutant deficient in hypoxanthine-guanine phosphoribosyltransferase and xanthine phosphoribosyltransferase validates the purine salvage pathway of *Leishmania donovani*. J Biol Chem. https://doi.org/10.1074/jbc.m600188200

Boitz JM, Ullman B (2013) Adenine and adenosine salvage in *Leishmania donovani*. Mol Biochem Parasitol. https://doi.org/10.1016/j.molbiopara.2013.06.005

Boitz JM, Strasser R et al (2012a) Adenine aminohydrolase from *Leishmania donovani*. Unique enzyme in parasite purine metabolism. J Biol Chem. https://doi.org/10.1074/jbc.m111.307884

Boitz JM, Ullman B et al (2012b) Purine salvage in Leishmania: complex or simple by design? Trends Parasitol 28(8):345–352. https://doi.org/10.1016/j.pt.2012.05.005

Brunschweiger A et al (2008) Selective nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) inhibitors: nucleotide mimetics derived from uridine-5′-carboxamide. J Med Chem. https://doi.org/10.1021/jm800175e

Cano-Soldado P, Pastor-Anglada M (2012) Transporters that translocate nucleosides and structural similar drugs: structural requirements for substrate recognition. Med Res Rev. https://doi.org/10.1002/med.20221

Carter NS et al (2000) Cloning of a novel inosine-guanosine transporter gene from *Leishmania donovani* by functional rescue of a transport-deficient mutant. J Biol Chem 275(27):20935–20941. https://doi.org/10.1074/jbc.M002418200

Carter NS, Landfear SM, Ullman B (2001) Nucleoside transporters of parasitic protozoa. Trends Parasitol 17(3):142–145. https://doi.org/10.1016/S1471-4922(00)01806-7

Carter NS et al (2010) Adaptive responses to purine starvation in *Leishmania donovani*. Mol Microbiol. https://doi.org/10.1111/j.1365-2958.2010.07327.x

Chawla B, Madhubala R (2010) Drug targets in Leishmania. J Parasit Dis. https://doi.org/10.1007/s12639-010-0006-3

Côrtes DF et al (2010) Low and high-dose intradermal infection with Leishmania major and *Leishmania amazonensis* in C57BL/6 mice. Mem Inst Oswaldo Cruz. https://doi.org/10.1590/s0074-02762010000600002

Cosentino-Gomes D, Meyer-Fernandes JR (2011) Ecto-phosphatases in protozoan parasites: possible roles in nutrition, growth and ROS sensing. J Bioenerg Biomembr 43(1):89–92. https://doi.org/10.1007/s10863-011-9334-y

Cummings HE et al (2012) Critical role for phosphoinositide 3-kinase gamma in parasite invasion and disease progression of cutaneous leishmaniasis. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.1110339109

Datta R et al (2005) Mutational analysis of the active-site residues crucial for catalytic activity of adenosine kinase from *Leishmania donovani*. Biochem J. https://doi.org/10.1042/bj20041733

Dayakar A et al (2012) A rapid method to assess the stage differentiation in *Leishmania donovani* by flow cytometry. Exp Parasitol. https://doi.org/10.1016/j.exppara.2012.09.006

de Almeida Marques-da-Silva E et al (2008) Extracellular nucleotide metabolism in *Leishmania*: influence of adenosine in the establishment of infection. Microbes Infect. https://doi.org/10.1016/j.micinf.2008.04.016

de Souza Leite M et al (2007) Trypanosoma brucei brucei: biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. Exp Parasitol. https://doi.org/10.1016/j.exppara.2006.09.002

de Souza MC et al (2010) The influence of ecto-nucleotidases on *Leishmania amazonensis* infection and immune response in C57B/6 mice. Acta Trop. https://doi.org/10.1016/j.actatropica.2010.04.007

Dick CF, Dos-Santos ALA, Meyer-Fernandes JR (2014) Inorganic phosphate uptake in unicellular eukaryotes. Biochim Biophys Acta Gen Subj. https://doi.org/10.1016/j.bbagen.2014.03.014

Dwyer KM et al (2007) CD39 and control of cellular immune responses. Purinergic Signal. https://doi.org/10.1007/s11302-006-9050-y

Eilers M et al (2000) Internal packing of helical membrane proteins. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.97.11.5796

El Fadili K et al (2005) Role of the ABC transporter MRPA (PGPA) in antimony resistance in *Leishmania infantum* axenic and intracellular amastigotes. Antimicrob Agents Chemother. https://doi.org/10.1128/aac.49.5.1988-1993.2005

Faleiro RJ et al (2014) Immune regulation during chronic visceral leishmaniasis. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0002914

Fonseca FV et al (2006) Trypanosoma rangeli: characterization of a Mg-dependent ecto ATP-diphosphohydrolase activity. Exp Parasitol. https://doi.org/10.1016/j.exppara.2005.09.005

Freitas-Mesquita AL, Meyer-Fernandes JR (2014) Ecto-nucleotidases and ecto-phosphatases from *Leishmania* and *Trypanosoma* parasites. Sub-Cell Biochem. https://doi.org/10.1007/978-94-007-7305-9_10

Fukuda Y, Schuetz JD (2012) ABC transporters and their role in nucleoside and nucleotide drug resistance. Biochem Pharmacol. https://doi.org/10.1016/j.bcp.2011.12.042

Galazka J et al (2006) Point mutations within the LdNT2 nucleoside transporter gene from *Leishmania donovani* confer drug resistance and transport deficiency. Int J Biochem Cell Biol. https://doi.org/10.1016/j.biocel.2005.12.016

García-Hernández R et al (2012) *Leishmania donovani* develops resistance to drug combinations. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0001974

Giarola NLL et al (2014) *Leishmania amazonensis*: increase in ecto-ATPase activity and parasite burden of vinblastine-resistant protozoa. Exp Parasitol. https://doi.org/10.1016/j.exppara.2014.08.013

Guimarães-Costa AB et al (2014) 3′-nucleotidase/nuclease activity allows Leishmania parasites to escape killing by neutrophil extracellular traps. Infect Immun 82(4):1732–1740. https://doi.org/10.1128/IAI.01232-13

Gupta S (2011) Visceral leishmaniasis: experimental models for drug discovery. Indian J Med Res 133:27–39

Hunsucker SA, Mitchell BS, Spychala J (2005) The 5′-nucleotidases as regulators of nucleotide and drug metabolism. Pharmacol Ther. https://doi.org/10.1016/j.pharmthera.2005.01.003

Kato H et al (2007) Identification and characterization of a salivary adenosine deaminase from the sand fly Phlebotomus duboscqi, the vector of Leishmania major in sub-Saharan Africa. J Exp Biol 210(Pt 5):733–740. https://doi.org/10.1242/jeb.001289

Landfear SM (2008) Drugs and transporters in kinetoplastid protozoa. Adv Exp Med Biol. https://doi.org/10.1007/978-0-387-77570-8_3

Landfear SM et al (2004) Nucleoside and nucleobase transporters in parasitic protozoa. Eukaryot Cell 3(2):245–254. https://doi.org/10.1128/ec.3.2.245

Leite PM et al (2012) Ecto-nucleotidase activities of promastigotes from Leishmania (Viannia) braziliensis relates to parasite

infectivity and disease clinical outcome. PLoS Negl Trop Dis 6(10):e1850. https://doi.org/10.1371/journal.pntd.0001850
Leprohon P et al (2015) Drug resistance analysis by next generation sequencing in Leishmania. Int J Parasitol Drugs Drug Resist. https://doi.org/10.1016/j.ijpddr.2014.09.005
Liu W et al (2006) Functional characterization of nucleoside transporter gene replacements in *Leishmania donovani*. Mol Biochem Parasitol. https://doi.org/10.1016/j.molbiopara.2006.09.002
Loeuillet C, Bañuls AL, Hide M (2016) Study of Leishmania pathogenesis in mice: experimental considerations. Paras Vectors. https://doi.org/10.1186/s13071-016-1413-9
Manzano JI, Castanys S (2013) A new ABC half-transporter in leishmania major is involved in resistance to antimony. Antimicrob Agents Chemother. https://doi.org/10.1128/aac.00211-13
Martin JL et al (2014) Metabolic Reprogramming during Purine Stress in the Protozoan Pathogen *Leishmania donovani*. PLoS Pathog 10(2):e1003938. https://doi.org/10.1371/journal.ppat.1003938
Maxwell MJ et al (2008) Proteomic analysis of the secretome of *Leishmania donovani*. Genome Biol. https://doi.org/10.1186/gb-2008-9-2-r35
Mondal S, Roy JJ, Bera T (2014) Generation of adenosine tri-phosphate in *Leishmania donovani* amastigote forms. Acta Parasitol 59(1):11–16. https://doi.org/10.2478/s11686-014-0203-9
Naderer T, Vince JE, McConville MJ (2004) Surface determinants of Leishmania parasites and their role in infectivity in the mammalian host. Curr Mol Med. https://doi.org/10.2174/1566524043360069
Ortiz D et al (2007) Molecular genetic analysis of purine nucleobase transport in Leishmania major. Mol Microbiol 64(5):1228–1243. https://doi.org/10.1111/j.1365-2958.2007.05730.x
Ortiz D et al (2009) An acid-activated nucleobase transporter from Leishmania major. J Biol Chem 284(24):16164–16169. https://doi.org/10.1074/jbc.M109.006718
Paes-Vieira L, Gomes-Vieira AL, Meyer-Fernandes JR (2018) NTPDase activities: possible roles on Leishmania spp infectivity and virulence. Cell Biol Int. https://doi.org/10.1002/cbin.10944
Paletta-Silva R et al (2011) *Leishmania amazonensis*: characterization of an ecto-3'-nucleotidase activity and its possible role in virulence. Exp Parasitol. https://doi.org/10.1016/j.exppara.2011.07.014
Paletta-Silva R et al (2012) *Leishmania amazonensis*: inhibition of 3′-nucleotidase activity by Cu^{2+}ions. Exp Parasitol. https://doi.org/10.1016/j.exppara.2012.03.001
Parodi-talice ÂA (2001) ABC transporters in the protozoan parasite Leishmania. Int Microbiol 4:159–166. https://doi.org/10.1007/s10123-001-0031-2
Pereira-Neves A et al (2014) Tritrichomonas foetus: characterisation of ecto-phosphatase activities in the endoflagelar form and their possible participation on the parasite's transformation and cytotoxicity. Exp Parasitol. https://doi.org/10.1016/j.exppara.2014.04.007
Peres-Sampaio CE et al (2008) *Leishmania amazonensis*: effects of heat shock on ecto-ATPase activity. Exp Parasitol. https://doi.org/10.1016/j.exppara.2008.01.003
Pinheiro CM et al (2006) *Leishmania amazonensis*: biological and biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. Exp Parasitol 114(1):16–25. https://doi.org/10.1016/j.exppara.2006.02.007
Podgorska M, Kocbuch K, Pawelczyk T (2005) Recent advances in studies on biochemical and structural properties of equilibrative and concentrative nucleoside transporters. Acta Biochim Pol. https://doi.org/10.1007/s00424-014-1510-6
Rai AK et al (2011) High concentration of adenosine in human visceral leishmaniasis despite increased ADA and decreased CD73′. Parasite Immunol 33:632–636. https://doi.org/10.1111/j.1365-3024.2011.01315.x
Rajasekaran R, Chen YPP (2015) Potential therapeutic targets and the role of technology in developing novel antileishmanial drugs. Drug Discov Today. https://doi.org/10.1016/j.drudis.2015.04.006
Ready PD (2014) Epidemiology of visceral leishmaniasis. Clin Epidemiol. https://doi.org/10.2147/clep.s44267
Robson SC, Se J, Zimmermann H (2006) The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance. Purinergic Signal 2:409–430. https://doi.org/10.1007/s11302-006-9003-5
Rodrigues V et al (2016) Regulation of immunity during visceral Leishmania infection. Parasit Vectors. https://doi.org/10.1186/s13071-016-1412-x
Saad-Nehme J et al (2004) A Mg-dependent ecto-ATPase is increased in the infective stages of Trypanosoma cruzi. Parasitol Res 93:41–50. https://doi.org/10.1007/s00436-003-1066-4
Sanchez MA et al (2004) 'Functional expression and characterization of a purine nucleobase transporter gene from Leishmania major. Mol Membr Biol 21(1):11–18. https://doi.org/10.1080/09687680310001400845
Sansom FM et al (2014) Golgi-located NTPDase1 of Leishmania major is required for lipophosphoglycan elongation and normal lesion development whereas secreted NTPDase2 is dispensable for virulence. PLoS Negl Trop Dis 8(12):e3402. https://doi.org/10.1371/journal.pntd.0003402
Sauvage V et al (2009) The role of ATP-binding cassette (ABC) proteins in protozoan parasites. Mol Biochem Parasitol. https://doi.org/10.1016/j.molbiopara.2009.05.005
Sindhu KJ, Kureel AK, Saini S, Kumari S, Verma P, Rai AK (2018) Characterization of phosphate transporter(s) and understanding their role in *Leishmania donovani* parasite. Acta Parasitol 63(1):75–88
Singh N, Kumar M, Singh RK (2012) Leishmaniasis: current status of available drugs and new potential drug targets. Asian Pac J Trop Med 5(6):485–497. https://doi.org/10.1016/S1995-7645(12)60084-4
Składanowski A (2013) The role of soluble 5′-nucleotidases in the conversion of nucleotide analogs: metabolic and therapeutic aspects. Curr Med Chem. https://doi.org/10.2174/09298673113320340005
Smith DF, Peacock CS, Cruz AK (2007) Comparative genomics: from genotype to disease phenotype in the leishmaniases. Int J Parasitol. https://doi.org/10.1016/j.ijpara.2007.05.015
Stauch A et al (2011) Visceral leishmaniasis in the indian subcontinent: modelling epidemiology and control. PLoS Negl Trop Dis. https://doi.org/10.1371/journal.pntd.0001405
Stein A et al (2003) Equilibrative nucleoside transporter family members from *Leishmania donovani* are electrogenic proton symporters. J Biol Chem 278(37):35127–35134. https://doi.org/10.1074/jbc.M306188200
Ullman B et al (2008) Purine and pyrimidine metabolism in Leishmania. Adv Exp Med Biol. https://doi.org/10.1007/978-0-387-77570-8_12
Valde R et al (2004) Transmembrane domain 5 of the LdNT1. 1 nucleoside transporter is an amphipathic helix that forms part of the nucleoside. Translocat Pathw 43:6793–6802
Van Den Berghe G, Vincent MF, Marie S (2012) Disorders of purine and pyrimidine metabolism. In: Inborn metabolic diseases: diagnosis and treatment. https://doi.org/10.1007/978-3-642-15720-2_37
Vasudevan G, Ullman B, Landfear SM (2001) Point mutations in a nucleoside transporter gene from *Leishmania donovani* confer drug resistance and alter substrate selectivity. Proc Natl Acad Sci. https://doi.org/10.1073/pnas.101537298

Vieira DP et al (2011) Leishmania chagasi: an ecto-3′-nucleotidase activity modulated by inorganic phosphate and its possible involvement in parasite-macrophage interaction. Exp Parasitol 127(3):702–707. https://doi.org/10.1016/j.exppara.2010.11.003

Weiss PHE et al (2015) Kinetic and biochemical characterization of Trypanosoma evansi nucleoside triphosphate diphosphohydrolase. Exp Parasitol. https://doi.org/10.1016/j.exppara.2015.03.009

World Health Organization (2010) Control of the leishmaniases. World Health Organization technical report series. https://doi.org/10.1038/nrmicro1766

Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta Mol Cell Res. https://doi.org/10.1016/j.bbamcr.2008.01.024

Young JD et al (2008) Human equilibrative nucleoside transporter (ENT) family of nucleoside and nucleobase transporter proteins. Xenobiotica. https://doi.org/10.1080/00498250801927427

Zimmermann H (2001) Ectonucleotidases: some recent developments and a note on nomenclature. Drug Dev Res. https://doi.org/10.1002/ddr.1097