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# Possible Effects of Microbial Ecto-Nucleoside Triphosphate Diphosphohydrolases on Host-Pathogen Interactions

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## INTRODUCTION

In 1972, the term "purinergic" was first used by Burnstock to describe signaling where ATP was the extracellular messenger molecule, which at the time was a rather radical idea (21). Since then the importance of purinergic signaling involving not just ATP but other nucleoside triphosphates (NTPs) and nucleoside diphosphates (NDPs) has become increasingly evident (20). It is now understood that purinergic signaling involves specific purinergic type 1 (P1) and type 2 (P2) receptors and is important in both neuronal and nonneuronal processes, including the modulation of inflammation and specific immune responses. P1 receptors, of which there are four subtypes, are activated by adenosine, which is generated by ecto-nucleotidases. In contrast, P2 receptors exist as two subtypes: P2X receptors, which are specific for ATP, and P2Y receptors, which are activated by ATP, ADP, UTP, UDP, ITP, and nucleotide sugars. P2X receptors are ligand-gated ion channel receptors, and seven subtypes have been identified, whereas P2Y receptors are G protein-coupled receptors that comprise eight known subtypes (20, 121).

Ecto-nucleoside triphosphate diphosphohydrolases (ecto-NTPDases) (gene family ENTPD) of the CD39 family are important ecto-nucleotidases that are characterized by the presence of five "apyrase conserved regions" (ACR1 to ACR5) and by the ability to hydrolyze NTPs and NDPs to the monophosphate form. Nucleoside monophosphates may then be catalyzed to nucleosides such as adenosine by the action of ecto-5′-nucleotidases (for example, mammalian CD73). Both of these ecto-enzymes are found on multiple cell types in a variety of eukaryotic organisms. The major function of these enzymes so far appears to involve purine salvage and the regulation of blood clotting, inflammatory processes, and immune reactions (69, 121, 151, 152). We and another group have

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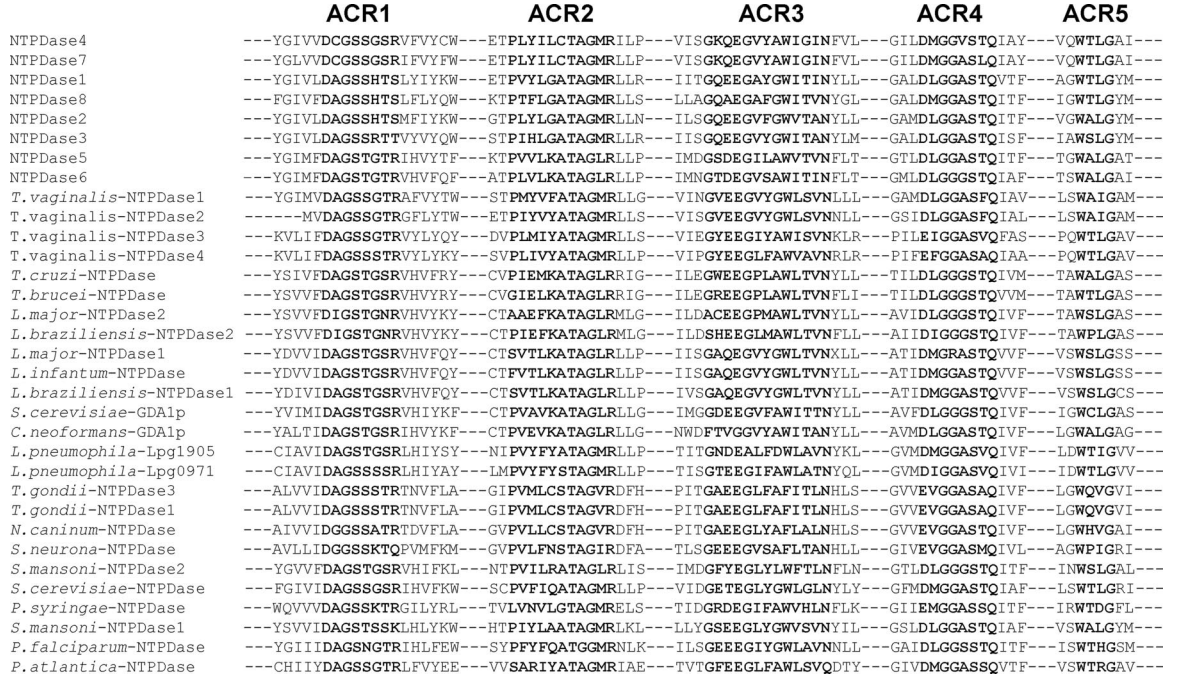


FIG. 1. Alignment of the amino acid sequences from ACR1 to -5 of known and hypothetical NTPDases present in eukaryotes and bacteria. Amino acid sequences were aligned using the ClustalW2 sequence alignment tool (http://www.ebi.ac.uk/Tools/clustalw2/).

recently shown that CD39 is expressed on regulatory T ($T_{reg}$) cells and mediates immune suppression (16, 39).

In mammals, NTPDases comprise at least eight members, with CD39/NTPDase 1, CD39L1/NTPDase 2, CD39L3/NTPDase 3, and NTPDase 8 located on the cell surface. Others are located on organelles and intracellular membranes or are secreted (14, 88, 121; H. Zimmermann, S. C. Robson, et al., presented at the Second International Workshop on Ecto-ATPases and Related Ecto-Nucleotidases, Maastricht, The Netherlands, 2000) (Fig. 1 and 2). The majority of these enzymes are membrane bound with the active site facing either the extracellular medium or the lumen of the organelle where they are sited (essentially communicating with the ecto-membranes), and are referred to as ecto-enzymes or ecto-NTPDases. An amino acid sequence alignment and phylogenetic analysis of NTPDases using the sequence analysis tool Clustal W (147) shows that the surface-located mammalian NTPDases are more closely related to each other than to mammalian NTPDases found in organelles (Fig. 1 and 3). NTPDases 5 and 6, which are intracellular but undergo secretion after heterologous expression (121), are also more closely related than NTPDases 4 and 7, which are entirely intracellular (Fig. 3).

Surface-located or secreted NTPDases in several important microbial pathogens of humans have also been described. The nomenclature surrounding these enzymes is complex, and since apyrases are classified by their ability to hydrolyze both ATP and ADP, here we have defined an NTPDase as a type of apyrase that contains the five ACRs and which is related to the CD39/NTPDase 1 family by amino acid sequence similarity. We recently characterized the first bacterial member of the NTPDase family, present in *Legionella pneumophila*, which enhances intracellular replication of the bacteria (123). Here we review our current knowledge of microbial NTPDases with an emphasis on those that have been directly implicated in virulence. We examine the potential role of these enzymes in the interaction of the pathogen with the immune system of the host and propose that microbial NTPDases, in addition to having potential roles in purine salvage, may subvert inflammatory and immune processes to modulate the host response to infection.

## MAMMALIAN NTPDases: REGULATION OF THROMBOSIS AND INFLAMMATION

The prototypic member of the NTPDase family, CD39, hydrolyzes ATP and ADP in an equivalent manner, with activity dependent on the presence of divalent cations, as is the case for all NTPDases. CD39 utilizes either $Ca^{2+}$ or $Mg^{2+}$ as a cofactor (83, 153). Recently the crystal structure of the extracellular domain of rat NTPDase 2 was solved (166). The structure identified several key amino acid residues in ACR1 to -5 that were variously involved in substrate and cofactor binding, although only ACR1 and ACR4 share homology with known ATP binding domains, namely, the actin-HSP 70-hexokinase β- and γ-phosphate binding motif (121, 166). In particular, E165 in ACR3 was important for the positioning of a water molecule for nucleophilic attack on the terminal phosphate of ATP or ADP. Several mutagenesis studies have confirmed the role of additional residues in ACR1 to -5 in the activity of mammalian NTPDases (49, 50, 68, 87, 126, 134, 135, 160). However, the level of catalytic activity and the specificity of NTPDases relate also to the membrane positioning of the protein, as demonstrated by the regulation of activity by the N- and C-terminal transmembrane domains of CD39 (67, 155).

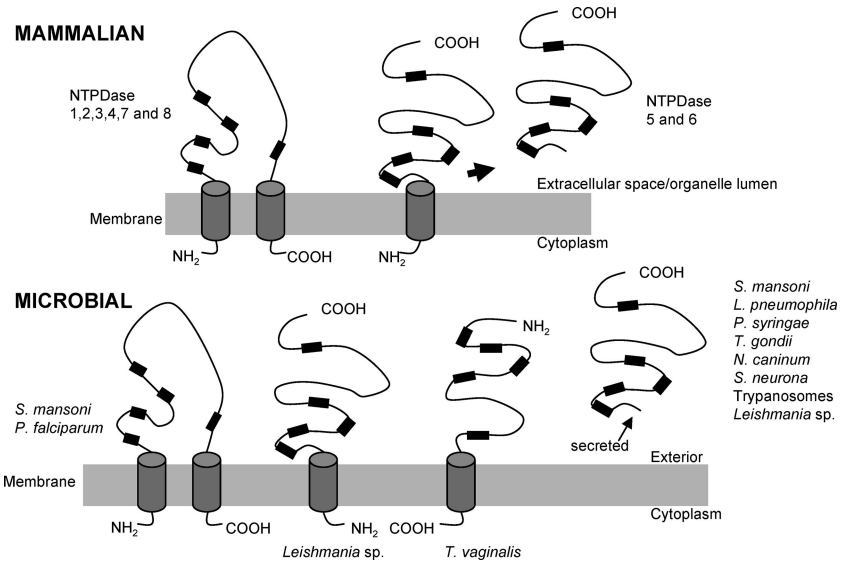


FIG. 2. Schematic representation of known and predicted localization of the NTPDases found in humans and pathogens, based on experimental evidence and sequence motifs, as listed in Table 1. Cylinders represent transmembrane domains, and black rectangles represent ACR 1 to -5. (Adapted from reference 168 with permission of the publisher. Copyright 2001 Wiley-Liss.)

Furthermore, NTPDases form homo-oligomers, and the state of oligomerization affects catalytic activity (67, 142, 155). CD39 contains a total of 11 cysteine residues (126), and the region between ACR4 and ACR5 in both CD39 and NTPDase 2 contains eight conserved cysteine residues. The cysteine residues play an important role in disulfide bond formation and oligomerization and have been shown to modulate substrate specificity (70, 166). Additionally, the N terminus containing the Cys13 residue of CD39 undergoes palmitoylation, which appears to contribute to the association of CD39 with lipid rafts (121). Cholesterol depletion therefore results in inhibition of CD39 activity (113). We recently showed that the N terminus of CD39 associates with the membrane scaffold protein RanBPM and that this association results in downregulation of catalytic activity (158).

CD39 is responsible for inhibiting platelet aggregation. The

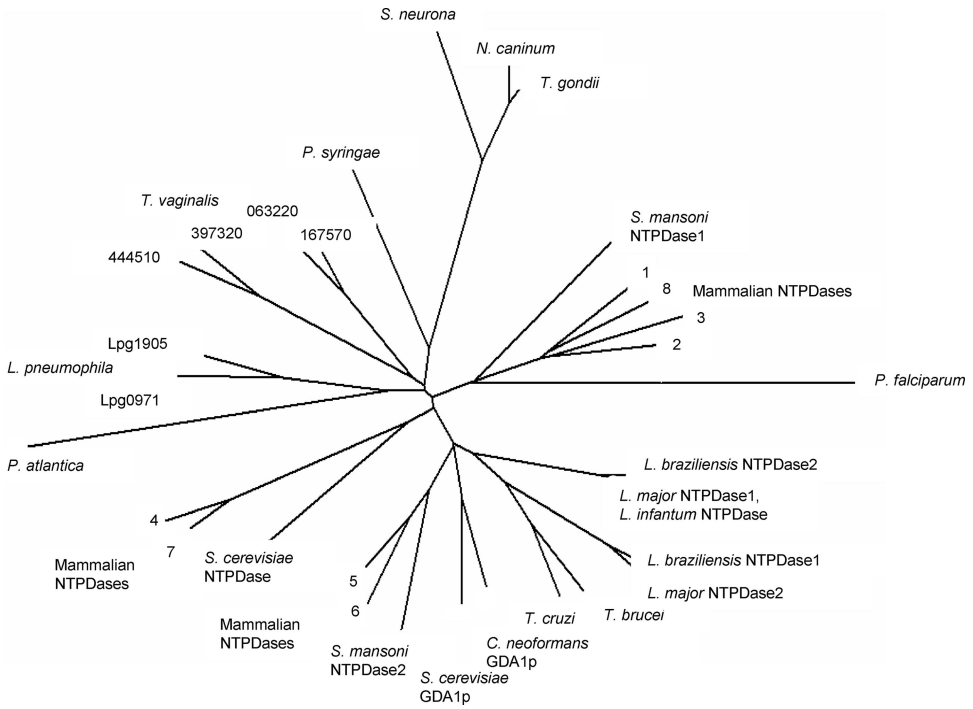


FIG. 3. Phylogenetic tree constructed from the amino acid sequence alignment in Fig. 1, with the resulting tree viewed and edited using the Phylodraw software. The accession numbers for the amino acid sequences used in the alignment are listed in Table 1.

rapid phosphohydrolysis of ADP released from activated platelets is thought to prevent the paracrine amplification of ADP-induced platelet recruitment and aggregation (65, 99). Furthermore, CD39 plays a role in modulating inflammation and the immune response by affecting the extracellular concentrations of ATP; this nucleotide serves as an immunostimulant of the immune system operative via P2 receptor pathways (105, 119). During acute inflammatory reactions, the bioactivity of CD39 is transiently but acutely compromised by oxidative stress (121), presumably resulting in a rise in extracellular ATP and increased activation of P2 receptors to stimulate host defenses.

### Effects on Vascular Homeostasis

Platelets possess three types of P2 receptors: the $P2X_1$ receptor, activated by ATP, and two P2Y receptors, $P2Y_1$ and $P2Y_{12,}$ which are both activated by ADP. Activation of $P2Y_1$ is responsible for initiation of aggregation, whereas activation of $P2Y_{12}$ is essential for completion of the ADP-induced aggregation response and for potentiation of platelet aggregation induced by other compounds such as thromboxaneA2. Activation of the $P2X_1$ receptor results in rapid calcium influx and contributes to platelet activation induced by low concentrations of collagen. Signaling through the $P2X_1$ receptor may also play a priming role in the subsequent activation of the $P2Y_1$ receptor by ADP (61).

In humans, NTPDase 2, which is expressed on pericytes and adventitial cells, stimulates platelet aggregation by preferentially hydrolyzing ATP to produce ADP, resulting in activation of $P2Y_1$ and $P2Y_{12}$ receptors (8). However CD39, which is dominantly expressed on the endothelium, hydrolyzes ADP to AMP, thus limiting platelet activation (8). CD39 is a true platelet modulator, as deletion of this gene in mice results in pericellular nucleotide flux that causes desensitization of $P2Y_1$ platelet receptors and a subsequent bleeding phenotype (51). Given the intrinsic role of CD39 and NTPDase 2 in the regulation of vascular homeostasis, it is possible that NTPDases produced by parasites, particularly by those parasites with extended bloodstream phases in their life cycles, also modulate platelet defense mechanisms to promote optimal parasite infection and growth.

### Modulation of the Host Inflammatory Response

ATP and other nucleotides such as UDP and UTP may be released from activated, stressed, or injured mammalian cells, resulting in a rise in the concentration of extracellular nucleotides, which function as a "danger signal" for the immune system. Extracellular ATP and other nucleotides then signal through P2 receptors to modulate the immune and inflammatory response in a variety of cell types, including immune and nonimmune cells (17, 20). In particular, extracellular ATP triggers the release of proinflammatory cytokines, including interleukin-1β (IL-1β) (55) and IL-2 and gamma interferon (93). NTPDase expression modulates this response by controlling the level of extracellular nucleotides. In addition, CD39 is highly expressed on activated $T_{reg}$ cells, which mediate immune suppression (16, 39). The hydrolysis of ATP by CD39 expressed on the surface of activated $T_{reg}$ cells functions first to control the cytolytic effects of high concentrations of ATP but also to control purinergic signaling and ensuing effects on the inflammatory process (16).

Interestingly, CD39 activity has been further linked to the inhibition of IL-1 release from endothelial cells (77), and the expression of CD39 on $T_{reg}$ cells has also been linked to decreased activation of dendritic cells (16). Therefore, the effect of NTPDases on the immune response is one of immune suppression, a process that may be mimicked by microbial NTPDases.

## NTPDases OF THE APICOMPLEXAN PARASITES AND TRYPANOSOMATIDS

The mechanisms by which pathogen-associated NTPDases may affect virulence have not been precisely elucidated. Several lines of experimental evidence suggest that hydrolysis of ATP and ADP by these enzymes has the potential to subvert and avoid host defense mechanisms. In addition, further hydrolysis of AMP to adenosine by either host or parasitic enzymes could have a number of effects, as adenosine plays an important role in limiting the inflammatory response and some pathogens may scavenge adenosine for growth (60).

### Purine Salvage and the Apicomplexan Parasites

The apicomplexan parasites, which comprise protozoa characterized by the presence of the unique organelle known as the apical complex, include a number of important human and animal pathogens, some of which are known to express NTPDases. These include *Toxoplasma gondii*, the causative agent of the zoonotic disease toxoplasmosis, which can have serious sequelae in pregnant women and immunocompromised people, and *Plasmodium falciparum*, the major cause of malaria, currently one of the most devastating infectious diseases worldwide. Apicomplexan parasites such as *T. gondii* and *P. falciparum* lack the ability to synthesize the purine ring de novo and instead rely on capturing purines from the host cell as an essential nutrient. The host purines are then transported into the parasite by nucleoside transporters and converted to purine nucleotides (44). Thus, the purine salvage pathway is vital to the metabolism of the parasites, and as such the enzymes and transporters involved in these pathways are attractive drug targets, provided that inhibitors of these components do not affect similar host enzymes. It has been suggested that the NTPDases known to be expressed by some parasites are involved in purine salvage, and therefore inhibitors specific for parasitic NTPDases have potential therapeutic uses (66). Here we discuss the NTPDases associated with the apicomplexan parasites and the evidence for a potential role in purine salvage, in addition to the other potential roles in pathogenesis such as the modulation of purinergic signaling.

***Toxoplasma gondii*.** *T. gondii* is capable of infecting both phagocytic and nonphagocytic cells (130), where it replicates within a parasitophorous vacuole that remains mostly sequestered from the endosomal network of the host cell (2, 33). Over 20 years ago, a highly active enzyme purified from *T. gondii* was shown to hydrolyze both ATP and ADP, although ADPase activity was only 18% of ATPase activity. It was designated an

NTP hydrolase (NTPase) and found to require activation by thiol compounds, presumably to induce essential disulfide bonds, similar to CD39 (5, 7).

Further work demonstrated that the purified NTPase was actually a mix of two isoenzymes, NTPase I and NTPase II, with identical molecular weights that differed in bioactivity but arose through gene duplication (6). In fact, three open reading frames encoding proteins with predicted NTPDase similarity are present in the genome of the virulent RH strain of *T. gondii*, and two are translated into proteins designated NTPase 1 and NTPase 3 (11), which correspond to the two isoenzymes NTPase II and NTPase I identified previously (6). The amino acid sequences of these two proteins show all five ACRs, and so the enzymes will be referred to as NTPDases from here on (6). Although both hydrolyze ATP, GTP, CTP, and UTP, NTPDase 3 has less than 1% of the relative activity of hydrolysis of ADP, GDP, CDP, and UDP but is 4.5 times more efficient at hydrolyzing ATP than NTPDase 1 (6). Interestingly, a 12-residue block of amino acids in the C termini of the NTPDase isoforms appears to dictate the substrate specificity. Through the synthesis of protein chimeras, amino acids FITG REMLASID and IVTGGGMLAAIN near the C termini of NTPDase 1 and NTPDase 3, respectively (residues 488 to 499), have been shown to affect specificity for NTPs and NDPs (107). This difference is also antigenically distinct, as sera from a small fraction of *T. gondii* patients can discriminate between NTPDase 1 and NTPDase 3 on the basis of these 12 residues (81).

In *T. gondii*, the NTPDases are present in dense granules in extracellular tachyzoites. Both proteins possess typical signal peptides for secretion, and following infection of the host cell, both NTPDases are secreted into the lumen of the parasitophorous vacuole (Fig. 1) (11, 130). As *T. gondii* is a purine auxotroph, the NTPDase activity may be part of a pathway that processes host cell nucleotides prior to uptake by the parasite (6, 130). Depletion of host cell ATP decreases the metabolism of the tachyzoites, leading to the suggestion that ATP is used by the parasite, either as energy for parasite processes or for purine salvage after processing by an NTPDase (137). However, *T. gondii* lacks a 5′-nucleotidase, the enzyme required to convert AMP to adenosine, suggesting that the NTPDases of *T. gondii* play an additional, more complex role than simply purine salvage, although it is also possible that *T. gondii* may exploit some host enzymes in the process of purine salvage (110).

NTPDase 3 is expressed in the actively replicating tachyzoite form of *T. gondii* but is downregulated in the dormant bradyzoite form of the parasite, which is involved in chronic host infection (108). The similarity of the genes encoding the NTPDases makes it difficult to create a specific mutant, and other evidence also suggests that the gene encoding NTPDase 3 is essential for viability (109). Antisense RNA studies showed that NTPDase 3 activity was necessary for intracellular replication but not for parasite invasion of the host cell (109). In contrast to that study, the pretreatment of parasites with a monoclonal antibody that recognized and inhibited both NTPDases resulted in decreased invasion of Vero cells, suggesting that NTPDase activity did contribute to invasion of host cells (84). NTPDase 3 is also indirectly implicated in virulence, as most virulent strains of *T. gondii* possess the gene but avirulent strains carry only the gene encoding NTPDase 1 (6, 107).

Apart from purine salvage, NTPDase activity may be directly related to virulence by influencing intracellular replication and exit from host cells (131). When NTPDase 3 is secreted by the parasite, it remains largely oxidized despite the reducing environment of the host cell. However, since parasitic infections generate host nitric oxide and other free radicals, which results in oxidative and nitrative stress (165), it may be that *T. gondii* itself is responsible for the reduction and therefore activation of NTPDase 3. Upon activation of the enzyme by exogenous thiols, the level of ATP in the host cell is rapidly depleted, and the parasites exit the host cell within a minute of thiol treatment (131). Exit of parasites in response to thiol compounds is $Ca^{2+}$ dependent, suggesting that ATP depletion releases $Ca^{2+}$ stores that are controlled by ATP (141). These data all suggest that the activation of a secreted NTPDase must be tightly regulated by the parasite (131). Recently, it has been shown that the reducing agent glutaredoxin (GRX) is secreted by the parasite as replication increases. GRX can activate NTPDase 3 in vitro, suggesting that GRX may be also secreted by the parasite to control the reduction and therefore activation of NTPDase 3, thereby stimulating exit from host cells (140). Therefore, either NTPDase 1 or NTPDase 3 activity has been implicated at each stage of parasite invasion, replication, and exit.

***Neosporum caninum*.** The genome of the closely related apicomplexan parasite *N. caninum* contains a single gene encoding a protein with 69% identity to *T. gondii* NTPDase that is most similar to NTPDase 3. Correspondingly, the *N. caninum* NTPDase can hydrolyze NTPs but not NDPs. The enzyme possesses a typical N-terminal signal peptide for secretion and is also localized to the dense granules of *N. caninum*, suggesting that it may be exported in a manner similar to that for the dense granule proteins of *T. gondii*, which are secreted via specific exocytosis (4, 32). While the role of this enzyme in pathogenesis is unclear, the inability of the enzyme to hydrolyze NDPs suggests that it is unlikely to play an independent role in purine salvage (110).

***Sarcocytis neurona*.** Another apicomplexan parasite related to *T. gondii* is *S. neurona*, which is a cause of equine myeloencephalitis. However, unlike *T. gondii*, it resides free in the cytoplasm of host cells during intracellular replication. The parasite also has demonstrable NTPDase activity, exhibiting hydrolysis of both ATP and ADP that is activated by thiol compounds. A single gene encoding a putative NTPDase is present in the genome of *S. neurona*, and specific polyclonal antibodies demonstrate that the protein is secreted into culture supernatant by parasites in vitro, which is consistent with the presence of a signal peptide. Other localization studies show that the NTPDase of *S. neurona* is absent during much of intracellular replication, but when present, it is apically localized and is detectable on newly invaded merozoites. It then disappears before reappearing on newly formed merozoites once they are completely mature but before exit from the host cell. This pattern of expression suggests the NTPDase is involved either in exit from host cells or in infection of new host cells, or possibly in both (167). Alternatively, it may be that the NTPDase is required when the parasite is extracellular, to

modulate host levels of extracellular ATP and perhaps protect against platelet activation and the immune response of the host.

***Plasmodium falciparum*.** NTPDase activity has not been described for the apicomplexan parasite *P. falciparum*, but a search of the genome of the virulent strain 3D7 shows the presence of an open reading frame encoding a predicted protein containing all five ACRs (64; B. Cooke, personal communication.). Similar to the case for CD39, the putative protein contains two predicted transmembrane domains, one located at the N terminus and the other at the C terminus, suggesting that the protein could be anchored in the membrane of the parasite with the active site facing the external medium. It is intriguing to note that whereas the NTPDases present in the other apicomplexan parasites appear to be closely related, the putative *P. falciparum* NTPDase appears to be evolutionarily distinct, suggesting that it may fulfill a different role in *P. falciparum* (Fig. 3).

## Trypanosomatids

***Trypanosoma* species.** *Trypanosoma* protozoa cause a range of diseases in both humans and animals, and NTPDase activity has been demonstrated in a number of species. The presence of $Mg^{2+}$-dependent ecto-ATPase activity was first demonstrated for *T. cruzi*, the causative agent of American trypanosomiasis, also known as Chagas' disease, a serious infection affecting the heart and gastrointestinal system which can be fatal (15, 57, 86). After entry into host cells, the vacuole containing *T. cruzi* undergoes lysosomal fusion, and the parasite subsequently escapes the vacuole and replicates inside the cytosol of the host cell (3).

The dependence of the ecto-ATPase activity on $Mg^{2+}$ suggested that the activity might be due to a member of the CD39/NTPDase family. Further evidence for the presence of an NTPDase in *T. cruzi* came from a second study that demonstrated a range of NTPDase activities in intact parasites and that anti-*T. gondii* NTPDase antibodies cross-reacted with a protein on the surface of the parasite (57). An open reading frame encoding a predicted secreted protein with similarity to NTPDases is present in the genome of *T. cruzi*, although it has not been determined if this gene does indeed encode the surface-located NTPDase identified by immunofluorescence. NTPDase activity is present in all forms of the parasite, although the activity of intact parasites at different developmental stages varies for infective trypomastigotes but not for noninfective epimastigotes (57). The infective trypomastigote also displays up to 20 times higher ecto-ATPase activity than the epimastigote stage (15). Both parasite stages are able to hydrolyze ATP and ADP, but the ratio of ATP to ADP hydrolysis for trypomastigotes is 2:1 while for epimastigotes it is 1:1. Epimastigotes have also been shown to hydrolyze GTP, GDP, UTP, and UDP, and the highest activity was observed against GTP (57).

Ecto-ATPase activity is inhibited by suramin and 4,4′-diisothiocyanostylbene 2′,2′-disulfonic acid, both of which are known inhibitors of NTPDase activity (15), Furthermore the presence of either inhibitor reduced the number of parasites attaching to and infecting mouse peritoneal macrophages. In contrast, the addition of 200 μM ATP resulted in approximately 30% more parasite-infected macrophages. The addition of suramin to epimastigotes also stimulated a fourfold increase in the ecto-ATPase activity of this parasite form, presumably in an effort to overcome the inhibition. These parasites were then much more capable of adhering to mouse macrophages than untreated epimastigotes (15). An additional study also demonstrated $Mg^{2+}$-dependent ecto-ATPase activity in *T. cruzi* and showed that ATPase activity is higher in the infective trypomastigote and amastigote stages than in the noninfective epimastigotes, again linking NTPDase activity to virulence (104).

Like the apicomplexan parasites, trypanosomes are unable to synthesize the purine ring (28), and the ability to hydrolyze NTPs and NDPs may be part of an essential nutrient salvage pathway (57). Additionally, it has been suggested that increased hydrolysis of ATP in the infective stage of *T. cruzi* may reflect modulation of the immune system by the parasite (57). ADPase activity may also be important for avoidance of host defenses, particularly since platelet recruitment results in the removal of opsonized *T. cruzi* from the circulation (149).

*Trypanosoma brucei* causes both African sleeping sickness in humans and nagana in livestock. Unlike *T. cruzi*, *T. brucei* is an extracellular pathogen that proliferates in the mammalian bloodstream (100). The genome of *T. brucei* encodes a putative secreted NTPDase, and assays using intact parasites demonstrate that *T. brucei* exhibits $Mg^{2+}$-dependent surface-located NTPDase activity that hydrolyzes ATP, GTP, CTP, UTP, and ADP. Interestingly, catalytic activity may be stimulated by other divalent cations, including zinc (47).

Another species, *Trypanosoma rangeli*, is capable of infecting humans and animals. Little is known about the biology of this parasite in vertebrate hosts, other than that it may cause disease following infection (136). *T. rangeli* also exhibits $Mg^{2+}$-dependent ecto-NTPDase activity that is highest against ATP, although ADP and other NTPs may also be hydrolyzed. NTPDase activity is stimulated by a number of carbohydrates, which has led to the suggestion that NTPDase activity may have a role in adhesion to the intermediate insect host, as carbohydrates on insect salivary glands play a part in adhesion by *Trypanosoma* species (59).

***Leishmania* species.** *Leishmania* parasites are responsible for a number of clinical syndromes known as visceral, cutaneous, and mucosal leishmaniasis, which are a consequence of parasite replication inside macrophages of the mononuclear phagocyte system, dermis, and naso-oropharyngeal mucosa, respectively. All of these syndromes have serious sequelae, but visceral leishmaniasis is particularly life-threatening (71). *Leishmania* replicates inside macrophages within a parasitophorous vacuole, the morphology of which varies between parasite species (23).

$Mg^{2+}$-dependent surface NTPDase activity has been detected in both *Leishmania tropica* and *Leishmania amazonensis* (13, 103), two of the species responsible for cutaneous leishmaniasis (71). However, as the genes encoding the proteins responsible for this activity have not been identified, it is not known if the proteins are actually members of the CD39/NTPDase 1 family. A BLAST search of the *Leishmania* genomes revealed that putative NTPDases containing the five ACRs are present in *Leishmania major*, *Leishmania infantum*, and *Leishmania braziliensis*, suggesting that it is likely that

other species of *Leishmania* also possess putative NTPDases. All the hypothetical proteins encoded by these *Leishmania* species possess either a predicted N-terminal transmembrane domains or an N-terminal signal peptide (Fig. 2; Table 1), suggesting that they may be anchored on the membrane surface of the parasite or secreted. Unlike many NTPDases, the enzymes identified in *L. tropica* and *L. amazonensis* cannot utilize $Ca^{2+}$ instead of $Mg^{2+}$ (13, 103). The enzymes also show different substrate specificities. The *L. tropica* enzyme can hydrolyze both ATP and ADP, although ADPase activity is only 21% of ATPase activity, and the enzyme also hydrolyzes other NTPs (103). Like that of a number of other parasite NTPDases, *L. tropica* NTPDase activity is stimulated by defined carbohydrates, namely, dextran sulfate, although it is unclear how the carbohydrates stimulate catalytic activity (116). In contrast to that of *L. tropica*, the *L. amazonensis* enzyme appears to utilize only NTPs as substrates (13). The location of the enzyme on the surface of *L. amazonensis* has been confirmed by immunogold electron microscopy using anti-CD39 antibodies, and this cross-reaction with anti-CD39 suggests that the protein is indeed a member of the CD39/NTPDase 1 family (118). Enzyme activity is highest toward the end of logarithmic replication and is higher in virulent strains than in avirulent strains. In addition, activity is increased more than 10-fold in the obligate intracellular amastigote stage compared to the promastigote stage (13, 118). Further evidence for a role in virulence comes from the observation that treatment with the anti-CD39 antibodies reduces the interaction of the parasites with mouse peritoneal macrophages (118). Finally, it was recently shown that ecto-ATPase activity of *L. amazonensis* is increased when the parasites undergo heat shock (115), which is of particular interest because it has been suggested that the heat shock response may play a role in invasion by parasites (120).

The high activity of the enzyme toward the end of parasite replication may indicate a role in exit from the infected cell, while the effect of the anti-NTPDase antibodies also suggests a role in host cell entry (118). In this context, the similarity of ACR1 and ACR4 to the actin-HSP 70-hexokinase β- and γ-phosphate binding motif is particularly curious (134). Phagocytosis of pathogens involves rearrangement of the actin cytoskeleton of the cell (101), and it may be that NTPDases are one of the pathogenic factors involved in disrupting normal phagocytic events and enabling intracellular pathogen such as *Leishmania* to establish their replicative niche. Additionally, it may reflect a need for the parasite to express the protein when extracellular in order to regulate host levels of extracellular ATP.

## NTPDase ACTIVITY IN OTHER PARASITES AND FUNGI

### *Schistosoma mansoni*

NTPDases have also been identified in the worm *Schistosoma mansoni*, the cause of intestinal schistosomiasis. *S. mansoni* is capable of surviving for several years in the human mesenteric vasculature by evading the host defenses, resulting in a chronic debilitating disease (151). *S. mansoni* possesses two isoenzymes containing the five ACRs that have NTPDase activity and which are encoded by two genes, SmATPDase 1 and SmATPDase 2 (45, 151). While SmATPDase 1 is located on the surface, SmATPDase 2 is secreted by the parasite (Fig. 2), suggesting distinct roles for the two enzymes (45, 96). As with many other NTPDases, activity is stimulated by $Ca^{2+}$ and $Mg^{2+}$ ions (148), and both ATP and ADP as well as other NTPs and NDPs are hydrolyzed (53). Since *S. mansoni* lives in the portal venous bloodstream, it has been postulated that ADPase activity prevents recruitment and aggregation of platelets, enabling the worm to avoid host hemostatic defenses (152). Recently, a new class of antischistosomal drugs, *N*-alkylaminoalkanethiosulfuric acids, were shown to partially inhibit tegumental *S. mansoni* NTPDase activity, suggesting that inhibiting the NTPDase activity of the parasite may compromise its survival (97).

### *Trichomonas vaginalis*

*Trichomonas vaginalis* is a flagellate protozoan that lives in the human urogenital tract. *T. vaginalis* is an extracellular pathogen that is cytotoxic for mammalian cells and the cause of trichomoniasis, a disease usually characterized by vaginitis (128). *T. vaginalis*-induced vaginitis is likely the most prevalent nonviral sexually transmitted disease worldwide (150). Comparison of the NTPDase activities of intact and disrupted *T. vaginalis* cells has demonstrated the presence of $Ca^{2+}$- or $Mg^{2+}$-dependent surface NTPDase activity that catalyzes the hydrolysis of ATP, ADP, and other nucleotides (40, 42). The enzyme(s) responsible for this observed activity has not been positively identified, but a search of the *T. vaginalis* G3 genome sequence revealed the presence of four genes encoding hypothetical proteins (locus tags TVAG_063220, TVAG_167570, TVAG_351590, and TVAG_397320) that all contain regions similar to the five ACRs found in NTPDases. This suggests that one or more of this class of enzyme is present in *T. vaginalis* and responsible for the NTPDase activity. Furthermore, the proteins encoded by these genes all possess predicted C-terminal transmembrane domains, implying that they are anchored in the membrane of the cell and function as ecto-enzymes (Fig. 2). D-Galactose, which is involved in adhesion of *T. vaginalis* to host cells, increases NTPDase activity by 90%, suggesting that the enzyme may play a role in adhesion, although to date no further evidence for this is available (42). Nevertheless, fresh isolates of *T. vaginalis* have increased surface NTPDase activity compared to a laboratory-adapted strain, suggesting a possible role in virulence (42, 146).

The concentration of free purine nucleotides in the vagina during disease can reach 10 mM as they are released from epithelial cells lysed during infection, and 90% of the nucleotides released are ATP (106). ATP itself is cytolytic toward mammalian cells not expressing NTPDase activity on their surface (58), but ATP does not lyse *T. vaginalis*, which may be a result of hydrolysis of ATP by the parasite. *T. vaginalis* is also a purine auxotroph, and so NTPDase activity may be part of a purine salvage pathway for the parasite (145). Aside from possessing higher ATPase and ADPase activities than laboratory-adapted strains, fresh isolates also exhibit variation in the ratio of ATP to ADP hydrolysis, suggesting they may have two or more NTPDases (146), as predicted by the *T. vaginalis* genome sequence.

The related parasite *Tritrichomonas foetus*, which inhabits

TABLE 1. Known and predicted members of the CD39/NTPDase 1 family in humans, parasites, and bacteria

| Organism | Protein | Accession no. | Size (amino acids) | Sequence motif(s)[a] | Predicted localization (% certainty)[b] | Actual localization | Evolutionary distance from CD39 | Preferred substrates | Reference(s) |
|---|---|---|---|---|---|---|---|---|---|
| Human | NTPDase 1 (CD39) | NP_001767 | 510 | 17–39 TMD, 477–499 TMD | Golgi apparatus (68.9) | Cell surface | | ATP, ADP, UTP, UDP | 91, 168 |
| | NTPDase 2 (CD39L1) | NP_982293 | 495 | 7–29 TMD, 461–483 TMD | ER[c] (81.4) | Cell surface | 1.07828 | ATP, UTP | 91, 168 |
| | NTPDase 3 (CD39L3) | NP_982293 | 529 | 24–46 TMD, 486–508 TMD | Golgi apparatus (62.6) | Cell surface | 1.23665 | ATP, ADP, UTP, UDP | 91, 168 |
| | NTPDase 4 | NP_004892 | 616 | 35–54 TMD, 561–583 TMD | ER (68.9) | Golgi apparatus | 2.38926 | NDPs (not ADP) | 154 |
| | NTPDase 5 (CD39L4) | NP_001240 | 428 | 1–20 SP, 5–22 TMD, 29–51 TMD | ER (81.4) | ER/secreted | 2.10482 | NDPs | 168 |
| | NTPDase 6 (CD39L2) | NP_001238 | 484 | 38–60 TMD | Golgi apparatus (71.5) | Cell surface/ secreted | 2.26930 | NDPs more than NTPs | 72, 161 |
| | NTPDase 7 (LALP1) | NP_065087 | 604 | 30–52 TMD, 548–570 TMD | ER (93.9) | Intracellular membrane compartment | 2.32551 | UTP, CTP, GTP | 129 |
| | NTPDase 8 | NP_001028285 | 495 | 1–34 SP, 13–35 TMD, 472–494 TMD | Lysosome (93.9) | Cell surface | 1.02653 | ATP, ADP, UTP, UDP | 88 |
| *T. gondii* | NTPDase 1 (NTPDase II) | Q27895 | 628 | 1–25 SP | Golgi apparatus (68.9) | Secreted | 2.86455 | NTPs, NDPs | 6, 11 |
| | NTPDase 3 (NTPDase I) | Q27893 | 628 | 1–25 SP | Golgi apparatus (75.1) | Secreted | 2.85459 | NTPs | 6, 11 |
| *N. caninum* | NTPDase | BAA31454 | 626 | 1–24 SP | Golgi apparatus (68.9) | Dense granules | 2.87507 | NTPs | 4 |
| *S. neurona* | NTPDase (SnNTP1) | AAP88692 | 714 | 1–19 SP | Golgi apparatus (81.4) | Secreted | 3.03931 | ATP, ADP | 167 |
| *P. falciparum* | NTPDase | AAN36910 | 827 | 43–61 TMD, 794–816 TMD, 93–116 CC | ER (62.6) | | 2.49665 | Unknown | 64 |
| *T. cruzi* | NTPDase | AAS75599 | 636 | 1–35 SP | Golgi apparatus (81.4) | Cell surface | 2.28283 | ATP, ADP, GTP, GDP, UTP, UDP | 57 |
| *T. brucei* | NTPDase | XP_847211 | 603 | 1–34 SP, 13–35 TMD, 68–103 CC | Golgi apparatus (75.1) | | 2.29964 | ATP, GTP, CTP, UTP, ADP | 45 |
| *L. major* | NTPDase 1 | XP_001681917 | 425 | 17–36 TMD | Golgi apparatus (68.9) | | 2.25424 | Unknown | 57 |
| | NTPDase 2 | CAJ02396 | 674 | 1–28 SP, 5–27 TMD, 70–94 CC | Golgi apparatus (81.4) | | 2.38343 | Unknown | 78 |
| *L. infantum* | NTPDase | XP_001464341 | 425 | 17–39 TMD | Golgi apparatus (71.5) | | 2.07866 | Unknown | 114 |
| *L. braziliensis* | NTPDase 1 | XP_001562178 | 425 | 1–32 SP, 20–42 TMD | Golgi apparatus (87.6) | | 2.07500 | Unknown | 114 |
| | NTPDase 2 | XP_001562788 | 691 | 1–35 SP, 19–41 TMD | Golgi apparatus (87.6) | | 2.38358 | Unknown | 114 |
| *L. amazonensis* | | | | | | | | ATP, UTP, CTP | 13 |
| *L. tropica* | | | | | | | | NTPs, ADP | 103 |
| *S. mansoni* | SmATPDase 1 | AAP94734 | 544 | 43–65 TMD, 508–530 TMD | Golgi apparatus (62.6) | Cell surface | 1.77270 | NTPs, NDPs | 53, 151 |
| | SmATPDase 2 | ABI79456 | 564 | None | ER (56.3) | Secreted | 2.34344 | NTPs, NDPs | 53 |
| *T. vaginalis* | 063220 | XP_001579703 | 441 | 403–425 TMD | ER (87.6) | | 1.98477 | NTPs, NDPs | 22 |
| | 167570 | XP_001298946 | 434 | 389–411 TMD | ER (62.6) | | 2.02683 | NTPs, NDPs | 22 |
| | 397320 | XP_001327224 | 458 | 1–23 SP, 410–432 TMD | ER (100) | | 2.34193 | NTPs, NDPs | 22 |
| | 444510 | XP_001325390 | 461 | 416–438 TMD | Golgi apparatus (81.4) | | 2.35400 | NTPs, NDPs | 22 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *S. cerevisiae* | NTPDase (YND1) | EDN62971 | 630 | 501–518 TMD | Golgi apparatus (68.9) | Golgi apparatus | 2.14165 | ADP,ATP, GDP, GTP, UDP, UTP | 63 |
| | GDA1p | NP_010872 | 518 | 1–28 SP, 7–24 TMD | Golgi apparatus (75.1) | Golgi apparatus | 2.18496 | GDP | 1, 159 |
| *C. neoformans* | GDA1p | AAR87384 | 661 | None | Golgi apparatus (56.3) | | 2.21608 | Unknown | G. Janbon, unpublished data |
| *L. pneumophila* | Lpg1905 | YP_095922 | 393 | 1–34 SP, 7–29 TMD | Unknown | Secreted | 2.22925 | ATP, ADP, GTP, GDP | 123, 124 |
| | Lpg0971 | YP_095005 | 381 | 1–20 SP, 331–353 TMD | Unknown | Secreted | 2.14919 | Unknown | 62, 123 |
| *P. syringae* | NTPDase | NP_793339 | 402 | 1–29 SP | Unknown | | 2.32661 | Unknown | 19 |
| *P. atlantica* | NTPDase | YP_663013 | 390 | None | Unknown | | 2.77708 | Unknown | Copeland et al., unpublished data |

[a] Sequence motifs were determined using the simple modular architecture research tool (SMART) program (95, 127). TMD, transmembrane domain; SP, signal peptide; CC, coiled coil.
[b] Localization prediction was performed using the pTARGET (eukaryotic proteins; http://bioapps.rit.albany.edu/pTARGET/) and PSORTB (bacterial proteins; http://www.psort.org/psortb/) programs.
[c] ER, endoplasmic reticulum.

the urogenital tract of cattle and may lead to abortion, also possesses $Mg^{2+}$-dependent NTPDase activity. The enzyme is unable to hydrolyze ADP but hydrolyzes ATP and other NTPs, and activity is stimulated by D-mannose and D-galactose, suggesting it may have a similar role in adhesion as postulated for *T. vaginalis* (80). For the NTPDases of both *T. foetus* and *T. vaginalis*, iron-depleted medium reduces the activity of the NTPDase, suggesting that iron may exert a positive regulatory role, although the significance of this is not yet known (43).

### Other Protozoa

*Entamoeba histolytica*, an enteric protozoan that causes invasive colitis and liver abscesses, also exhibits $Mg^{2+}$-dependent NTPDase activity that catalyzes the hydrolysis of ATP and to a lesser extent ADP, with still lower activity rates for other NTPs (10). Pathogenic *E. histolytica* has a higher rate of activity than nonpathogenic *E. histolytica* or free-living *Entamoeba moshkovskii*, suggesting a role in virulence. Additionally, D-galactose stimulates NTPDase activity, as for a number of other parasitic NTPDases (10). A second amoebic pathogen, *Acanthamoeba*, can cause fatal encephalitis and keratitis in humans, and the amoebae show ecto-ATPase activity, which is dependent on divalent cations and inhibited by the NTPDase inhibitor suramin (133). Increased enzyme activity is associated with virulent isolates rather than environmental isolates, again suggesting a role in host-pathogen interactions. *Acanthamoeba* binds to endothelial cells using a mannose binding protein, and it has been shown that alpha-mannose stimulates enzyme activity. Furthermore, suramin inhibition of enzyme activity results in decreased cytotoxicity and binding to host cells, all supporting a role in virulence (133).

While the enzyme activities detected for *Acanthamoeba* and *Entamoeba* species appears to indicate the presence of NTPDases, a BLAST (tblastn) search of the genomes of both *Acanthamoeba castellanii* and *E. histolytica* using the CD39 amino acid sequence did not reveal any significant homologues containing five ACRs. Although these studies support the hypothesis that ecto-ATPase activity is related to the virulence of many parasites, it seems probable that the observed catalytic activities are due to enzymes unrelated to the CD39/NTPDase 1 family.

Most recently, $Mg^{2+}$-stimulated ecto-ATPase activity was demonstrated in *Giardia lamblia*, a flagellated protozoan that can cause small intestinal diarrhea (46). Hydrolysis of ADP and AMP was also observed, but $Mg^{2+}$ did not stimulate this activity. BLAST searching of the *G. lamblia* genome using the CD39 amino acid sequence also failed to reveal any NTPDase homologues, suggesting that this ecto-enzyme activity is not due to a member of the NTPDase family. Furthermore, the role of this enzymatic activity in the virulence of the parasite is currently unknown.

### Fungi

The nonpathogenic yeast *Saccharomyces cerevisiae* possesses membrane-bound NTPDases containing all five ACRs that are capable of hydrolyzing NTPs and NDPs. These enzymes are not surface located and are involved in Golgi glycosylation processes (12, 63). Accordingly, they are evolutionarily more

closely related to the mammalian NTPDases 4, 5, 6, and 7, which are localized in the cytoplasm of the cell (Fig. 3). Surface NTPDase activity was recently described for the pathogenic yeast *Cryptococcus neoformans*, an important cause of pneumonia and meningoencephalitis, particularly in immunocompromised individuals. The NTPDase activity was stimulated by $Mg^{2+}$ and showed high rates of ATP, ITP, GTP, CTP, and UTP, but not ADP, hydrolysis (82). The genome of *C. neoformans* encodes a hypothetical protein containing all five ACRs, but this is predicted by pTARGET to be Golgi localized, so it is unclear if the observed enzyme activity is due to a member of the CD39/NTPDase 1 family or an unrelated enzyme. It is worth noting however, that the predicted localization of these enzymes often does not correspond with the known localization (Table 1). Ecto-ATPase activity was also recently demonstrated for the pathogen *Fonsecaea pedrosoi*, the cause of chromoblastomycosis, a chronic, subcutaneous fungal infection (29). Enzyme activity was stimulated by $Mg^{2+}$ and was postulated to play a role in fungal physiology and/or pathogenesis (29). The protein(s) responsible for this activity is also yet to be identified.

## *LEGIONELLA PNEUMOPHILA*: THE PROKARYOTIC PUZZLE

Expression of the CD39/NTPDase 1 family of enzymes is extremely rare in prokaryotes. These ecto-enzymes appear to have been acquired only upon association with eukaryotes. While CD39/NTPDase 1 family members are present in all higher eukaryotes, the presence of the enzymes in lower eukaryotes is variable and not necessarily consistent with the putative phylogenetic relationships and evolutionary development of eukaryotic organisms. Therefore, it remains unclear when and why the CD39/NTPDase 1 family of enzymes evolved.

Recently, we characterized the first prokaryotic NTPDase in *Legionella pneumophila*, the major causative agent of Legionnaires' disease. Legionnaires' disease is a systemic disease characterized by a severe pneumonia and possible renal impairment which may be fatal even with appropriate antibiotic therapy (38, 123). Key to the pathogenesis of Legionnaires' disease is the ability of *L. pneumophila* to replicate inside cells, in particular alveolar macrophages. The innate capacity of *L. pneumophila* and other species of *Legionella* to replicate inside eukaryotic cells is widely attributed to their long-standing association with environmental protozoa (143). *L. pneumophila* is a parasite of many protozoan species, particularly free-living amoebae such as *Acanthamoeba*, *Hartmanella*, and *Naegleria* species, which also serve as useful infection models to study *L. pneumophila* intracellular replication (56). In both mammalian cells and amoebae, the *L. pneumophila* vacuole avoids the endocytic pathway and interacts instead with the exocytic pathway of the cell so that the replicative vacuole ultimately develops characteristics of the endoplasmic reticulum (75, 76).

Possible DNA transfer events between the bacteria and their environmental eukaryotic hosts may have facilitated the acquisition of genes encoding proteins that mimic eukaryotic signaling and protein interaction motifs, thereby allowing *Legionella* to interfere with eukaryotic signaling and trafficking pathways. Indeed the *L. pneumophila* genome encodes a surprising abundance of proteins that harbor eukaryotic motifs (18, 25, 41).

Two genes, annotated as lpg1905 and lpg0971 in the *L. pneumophila* Philadelphia genome, each encode predicted proteins that contain all five ACRs typical of eukaryotic NTPDases (26). The presence of NTPDase genes in *L. pneumophila* is surprising since the lower environmental eukaryotes that *Legionella* typically associates with, such as amoebae, do not possess CD39/NTPDase 1 enzymes. Therefore, although it is widely assumed that *L. pneumophila* coevolved with free-living protozoa and acquired many eukaryotic-like determinants from this relationship (41), in this case it is not clear which eukaryote was the source of the *L. pneumophila* NTPDases.

Lpg1905 is a functional NTPDase, able to hydrolyze both ATP/ADP and GTP/GDP with similar efficiencies (123, 124). Lpg1905 is secreted by the bacterium in vitro, although its site of action during infection is unclear. The enzyme has maximal activity at neutral pH (124), and given that the purpose of *Legionella* virulence determinants is to enhance bacterial replication in amoebae, the enzyme presumably has an intracellular function. Indeed, inactivation of lpg1905 results in defective replication of *L. pneumophila* within amoebae, epithelial cells, and macrophages (123). Substantial levels of enzyme activity are also required for full virulence of *L. pneumophila* in an A/J mouse model of Legionnaires' disease, although it is not clear if it is ATP/ADPase or GTP/GDPase activity or both that contribute to *L. pneumophila* infection (124). The second putative secreted NTPDase, Lpg0971, is dispensable for replication within amoebae and macrophages (123) but contributes to virulence in the A/J mouse lung infection model, similar to Lpg1905 (F. M. Sansom et al., unpublished data). Although it is not yet proven that Lpg0971 possesses NTPDase activity, the additional requirement of lpg0971 for lung infection suggests that the enzymes may mediate effects on the host response over and above any role in intracellular replication of *L. pneumophila*. The contribution of NTPDases to *L. pneumophila* virulence and replication is unlikely to relate to purine scavenging, as *L. pneumophila* is not purine auxotroph (117). Rather, it may relate to effects on host nucleotide levels and subsequent effects on P2 receptor signaling.

A putative NTPDase is also annotated in the genome of the plant pathogen *Pseudomonas syringae* pv. *tomato* strain DC3000 (locus tag PSPTO_3560) (19). The predicted protein possesses a signal peptide for secretion but to date remains uncharacterized. Finally, a BLAST search of all known bacterial sequences revealed only one other putative NTPDase, an uncharacterized hypothetical protein containing all five ACRs (locus tag Patl_3457) encoded in the genome of the marine bacterium *Pseudoalteromonas atlantica*, which is a pathogen of crabs (34; A. Copeland, S. Lucas, A. Lapidus, K. Barry, J. C. Detter, T. Glavina del Rio, N. Hammon, S. Israni, E. Dalin, H. Tice, S. Pitluck, E. Saunders, T. Brettin, D. Bruce, C. Han, R. Tapia, P. Gilna, J. Schmutz, F. Larimer, M. Land, L. Hauser, N. Kyrpides, E. Kim, A. C. Karls, D. Bartlett, B. P. Higgins, and P. Richardson, unpublished data).

## CD39 EXPRESSION AND CAPTURE BY HIV

Increased expression of CD39, with a concomitant increase in observed NTPDase activity, occurs in lymphocytes isolated from patients infected with human immunodeficiency virus (HIV) (94). In addition, it now appears that CD39 is incorporated into HIV virions, where it remains enzymatically active (9). The CD39-bearing virions can inhibit platelet aggregation, although the importance of this in pathogenesis is unclear, as severe bleeding is not common in HIV/AIDS patients (9). Enhanced CD39 expression may alternatively have effects on extracellular ATP concentrations and purinergic signaling, in a manner similar to that for other pathogens bearing surface-located NTPDases, although this remains to be proven.

## PURINERGIC SIGNALING AND THE HOST-PATHOGEN INTERACTION

### Interference with P2 Receptor Signaling by Parasites

**Possible effects on vascular homeostasis.** The expression of NTPDases on the surface of pathogens that invade the bloodstream has the potential to alter platelet activity. Hydrolysis of ATP and ADP by NTPDases may influence the activation of $P2X_1$, $P2Y_1$, and $P2Y_{12}$ receptors, resulting in interference with normal thrombotic defense mechanisms. This is particularly relevant to the metazoan parasite *S. mansoni* as it enters the bloodstream. Experimental percutaneous infection of mice with *S. mansoni* results in a transient thrombocytopenia 2 days after infection, at about the stage that the organism reaches the bloodstream, suggesting that platelet activation and attachment of platelets to larvae is a host defense mechanism against infection (139). This is supported by the observation that platelets can attach to larvae in vitro. However, a few days after infection, platelet levels are similar in infected and control animals, suggesting that the larvae become resistant to platelet defense mechanisms (139). Since the larval forms of *S. mansoni* express both SmATPDase 1 and SmATPDase 2 (45, 96), it is possible that upon entry of the parasite into the bloodstream, exposure to platelets leads to increased expression of the enzymes. The subsequent hydrolysis of ADP and limitation of platelet activation could then result in resistance of the parasite to platelet defense mechanisms.

Another parasite with a significant bloodstream phase is *P. falciparum*, and while suppression of platelet aggregation in humans infected with *P. falciparum* has been reported (138), other work has suggested that *P. falciparum* infection enhances platelet aggregation (112). Even though the precise effect of the parasite on platelets is unclear, the presence of a putative NTPDase gene in the genome of *P. falciparum* warrants further investigation. In particular, determining if the protein is located on the parasite surface and what, if any, preference the enzyme displays for ATP and/or ADP would assist in clarifying the possible effect of *P. falciparum* on platelet activation during infection.

The life cycles of both African and American trypanosomes involve blood-borne stages, and it has been suggested that the NTPDases on the surface of the parasites are involved in inhibiting platelet recruitment (57, 99). In the case of *T. brucei*, where the parasite remains extracellular and replicates within the bloodstream, the ability to inhibit platelet defense mechanisms would be highly advantageous. However, it is difficult to reconcile this with the fact that both *T. brucei* and *T. cruzi* are known to cause platelet aggregation and resultant thrombocytopenia (111, 144). One explanation may be that in all three trypanosome species in which NTPDases have been studied, the enzymes hydrolyze ATP preferentially, resulting in liberation of ADP and transient platelet activation. Although preferential ATPase activity would provide ADP for activation of $P2Y_1$ and $P2Y_{12}$ receptors, ADP hydrolysis by these enzymes is nevertheless still relatively efficient (47, 57, 59). Again, more work is needed to clarify the contribution of NTPDases to platelet responses during infection.

**Modulation of the host inflammatory response.** Extracellular ATP and other nucleotides constitute potent "danger signals" for the host immune and inflammatory responses (17). ATP in particular can trigger the release of proinflammatory cytokines such as IL-1β through P2 receptor signaling (93). Extracellular ATP also activates dendritic cells and induces secretion of IL-12 (125). CD39 has been shown to inhibit ATP-stimulated cytokine release from mammalian cells (77), and the expression of CD39 on $T_{reg}$ cells has also been linked to decreased activation of dendritic cells (16). Therefore the hydrolysis of extracellular nucleotides by microbial NTPDases could potentially mimic the action of mammalian NTPDases by influencing the immune response during infection, including the induction of inflammatory responses, $T_{reg}$ cell activation, and/or dendritic cell maturation. Interestingly, *Leishmania*, *T. cruzi*, and *T. gondii* have all been shown to inhibit IL-12 production by dendritic cells (122), and the invasion of dendritic cells by live *T. gondii* does not result in dendritic cell maturation (102). Similarly, dendritic cells pulsed with live *L. pneumophila* do not undergo phenotypic maturation and secrete lower levels of cytokines (85). Nevertheless, despite this link, it remains to be seen if microbial NTPDases can function in a manner similar to that for CD39 in vivo.

### Interference with P2 Receptor Signaling by Bacteria

Extracellular ATP has already been shown to be involved in host defense mechanisms during certain bacterial infections (52, 92). Although in general bacterial pathogens do not possess CD39/NTPDase 1 enzymes, several organisms have nonetheless been shown to interfere with ATP-mediated signaling by other means (162, 163). For example, extracellular ATP appears to stimulate killing of intracellular *Mycobacterium bovis* and *Mycobacterium tuberculosis* by infected human macrophages in a manner dependent on $P2X_7$ receptors (52, 92). *Mycobacterium* spp. are intracellular pathogens of macrophages that inhibit phagosome maturation as a path to intracellular replication. Stimulation with extracellular ATP overcomes the inhibition of phagolysosome fusion and drives maturation of the *Mycobacterium* vacuole to a mature phagolysosome where the bacteria are killed. Interestingly, a recent study showed that extracellular ATP does not induce the killing of intracellular *Mycobacterium avium* subsp. *paratuberculosis* in bovine mononuclear phagocytes, demonstrating that species differences do exist (157). Although the exact mechanism behind ATP-induced killing of *Mycobacterium* is unknown, polymorphisms in the $P2X_7$ receptor are associated with increased susceptibility to the dissemination of mycobacterial

growth and clinical disease (54). *M. bovis* is known to secrete at least two enzymes that alter ATP concentration, an ATPase and a NDP kinase (Ndk). Filtered culture supernatants prevent ATP-induced macrophage death, although the initial study describing this result did not demonstrate which enzyme(s) secreted by the bacteria was responsible for this protective effect (163). More recently, purified Ndk secreted by *M. tuberculosis* was shown to have ATPase and GTPase activities. Surprisingly, the same study also demonstrated that Ndk actually enhanced ATP-induced cytotoxicity in macrophages, which was inhibited in the presence of a P2 receptor antagonist (27). As Ndk catalyzes the transfer of the terminal phosphate group of NTPs to NDPs, the authors postulated that this effect on cytotoxicity resulted from Ndk-mediated conversion of ATP into other nucleotides that act as better agonists toward P2 receptors, although there is no direct evidence for this hypothesis.

Recently, the intracellular pathogenic bacterium *Porphyromonas gingivalis* was shown to interfere with $P2X_7$ receptor-mediated apoptosis in epithelial cells (162). While *P. gingivalis* does not possess an NTPDase, it also secretes an Ndk into culture supernatants. In contrast to the case for *M. tuberculosis*, the secretion of this enzyme is associated with the inhibition of $P2X_7$-mediated apoptosis, suggesting that Ndk secreted from different pathogens results in varied effects on the host. The reasons for this are not understood, but it may result from differences in other nucleotide concentrations or other, as-yet-unknown factors. *Pseudomonas aeruginosa* also secretes several ATP-utilizing enzymes (including Ndk and an ATPase). The presence of purified ATPase or Ndk reduces the cytotoxicity associated with ATP-induced $P2X_7$ receptor activation and the subsequent loss of macrophage viability. This effect presumably results from hydrolysis of ATP by either enzyme and/or sequestration of ATP from the $P2X_7$ receptors (164).

Extracellular ATP (but not other nucleotides) when incubated with macrophages infected with *Chlamydia trachomatis* reduces the number of infectious bacteria within the macrophages. However, the bacteria themselves appear to also interfere with apoptosis by decreasing the activity of the $P2X_7$ receptor (35), although the mechanism by which this occurs is unclear. As is the case for *M. tuberculosis*, treatment of infected macrophages with extracellular ATP results in the death of bacteria from activation of phospholipase D in a manner dependent on activation of $P2X_7$ receptors (36).

*Mycoplasma hominis*, an extracellular organism that colonizes the human urogenital tract, also possess an ecto-ATPase, although this enzyme is not a member of the NTPDase family (74). Surprisingly, a recent study demonstrated that this enzyme was able to induce apoptosis, and although the mechanism by which this occurred was not elucidated, it was postulated that it may be due to the generation of ADP and/or other breakdown products of ATP, again suggesting that ATP-utilizing enzymes of pathogens may influence purinergic signaling (73).

While none of these bacteria possess a typical NTPDase of the CD39/NTPDase 1 family, the degradation of extracellular ATP by pathogens has a proven effect on host cell viability and the capacity of the host to clear an infection. Therefore, microbial NTPDases are likely to interfere with similar processes in pathogen-infected macrophages. In particular, the observation in a number of studies that secreted ATP-utilizing enzymes from intracellular pathogens interfere with purinergic signaling strongly suggests a similar role for the secreted and surface-located NTPDases expressed by the microbial pathogens reviewed here.

## Adenosine Generation by Pathogens

The immune suppression elicited by $T_{reg}$ cells also relies on the concurrent expression of CD73 by these cells (39). CD73 is an ecto-5′-nucleotidase that hydrolyzes AMP to produce adenosine, a molecule that signals through P1 receptors, specifically A2A receptors, to induce a number of immunosuppressive effects such as the inhibition of effector T-cell activation and suppression of proinflammatory cytokine expression (39). Therefore, the immunosuppressive effect of CD39 results not only from the inhibition of P2 receptor signaling by the degradation of ATP but also from the activation of P1 receptors by adenosine.

Ecto-5′-nucleotidase activity has also been detected on the surface of *T. vaginalis* (145). While the parasite is a purine auxotroph and may utilize some of the adenosine produced for growth, free adenosine may also play a secondary role as a mediator of immune suppression. The trypanosomes *Leishmania* and *S. mansoni* have all been shown experimentally to hydrolyze AMP to generate adenosine at the surface of the parasite (13, 47, 118, 132), suggesting a similar role for the enzyme in these organisms. Of particular interest is the observation that C57BL/6 mice are able to control infection by *L. braziliensis* but develop chronic lesions when infected with *L. amazonensis* (98). The *L. amazonensis* parasites exhibit higher levels of AMP hydrolysis, thus producing adenosine in larger amounts, and mice infected with *L. amazonensis* show decreased production of proinflammatory cytokines, thereby raising the possibility that adenosine production by *Leishmania* parasites contributes to immune suppression.

In contrast, apicomplexan parasites are not known to possess ecto-5′-nucleotidases (110), so it is unclear if adenosine is generated by these parasites. Similarly, *L. pneumophila* does not appear to possess an ecto-5′-nucleotidase (24, 26) and does not reportedly hydrolyze AMP. Although to date there is no evidence of adenosine generation by the apicomplexan parasites and *Legionella*, it is possible that host enzymes such as CD73 could theoretically mediate the hydrolysis of excess AMP resulting from the action of microbial NTPDases.

## Nonadenine Nucleotide Signaling

It is interesting that of the microbial NTPDases described so far, most hydrolyze purine and pyrimidine bases such as GTP, UTP, and CTP with efficiencies similar to those for ATP and ADP. Unusually, Lpg1905 from *L. pneumophila* hydrolyzes only ATP/ADP and GTP/GDP and shows very limited activity against CTP/CDP and UTP/UDP (124). The NTPDases that do efficiently hydrolyze nonadenine nucleotides include those found in the apicomplexan parasites *T. gondii* (6) and *N. caninum* (4) and those found in *T. vaginalis* (40), *T. foetus* (80), the trypanosomes (47, 57, 59), and *S. mansoni* (53). In addition to their activity against NTPs, the NTPDases from *T. gondii* (6), *T. vaginalis* (40), *T. cruzi* (57), and *S. mansoni* (53) hydrolyze

other NDPs. Nonadenine NTPs and NDPs such as UTP and UDP are agonists for a number of P2Y receptors and in some cases have higher affinity for these receptors than ATP (20). UTP has also been shown to stimulate expression and release of the proinflammatory cytokine IL-6 (48, 89). Furthermore, UDP, a selective P2Y6 agonist, stimulates production and release of IL-8 and tumor necrosis factor alpha in human monocytic cell lines (37, 156). Extracellular nucleotides are also involved in lipopolysaccharide-induced neutrophil migration (90). Therefore, the microbial NTPDases may potentially utilize several substrates to interfere with P2 receptor signaling.

## CONCLUSIONS

In recent years the identification of an increasing number of human pathogens with secreted and plasma membrane-associated NTPDase activity has raised a number of intriguing questions regarding the role in of these enzymes in interactions with the mammalian host. The similarities between microbial and mammalian NTPDases suggest that pathogens have the capacity to interfere with pathways mediated by host NTPDases, such as modulation of vascular homeostasis and inflammatory and immune responses. While further work is required to elucidate the mechanism(s) by which microbial NTPDases influence virulence, it is evident from the work reviewed here that investigating the effects of these enzymes on purinergic signaling may yield valuable information.

### NTPDases as Possible Targets for Antimicrobial Therapy

In addition, the characterization of crucial differences between the microbial NTPDases and mammalian NTPDases may lead to the development of selective inhibitors that target microbial survival and enhance antimicrobial immune responses. The fact that the enzymes have an external location, are implicated in parasite survival, and are widely distributed among different eukaryotic human pathogens make them appealing targets for the development of antimicrobial agents. However, the success of microbial NTPDases as pharmacological targets will depend on the identification of inhibitors that are selectively toxic for microbial pathogens but have little effect on the mammalian enzymes. Some promise has been shown already with the new *N*-alkylaminoalkanethiosulfuric acid class of antischistosomal drugs that partially inhibit parasite NTPDase activity (54).

We recently observed that Lpg1905 from *L. pneumophila* exhibited different sensitivities to inhibition by novel polyoxometalate NTPDase inhibitors than mammalian NTPDases (124). Although these differences were not pharmacologically useful, this demonstrated that there are likely to be structural differences between the enzymes that could be exploited for inhibitor development.

The importance of protein structures for understanding enzyme function and for the development of NTPDases as targets for antimicrobial therapy is paramount. The rational design of inhibitors that have greater potency against the microbial enzymes than against the host will depend on identifying structural differences between the microbial and mammalian enzymes. The recent elucidation of the crystal structure of rat NTPDase 2 will form an important basis for studying structural differences within this family of enzymes (166). This work needs to take place in concert with detailed pathogenesis studies of the role of microbial NTPDases in infection so that we can approach a clear understanding of the contribution of these enzymes to parasite survival and host immunity.

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## REFERENCES

1. **Abeijon, C., P. Orlean, P. W. Robbins, and C. B. Hirschberg.** 1989. Topography of glycosylation in yeast: characterization of GDPmannose transport and lumenal guanosine diphosphatase activities in Golgi-like vesicles. Proc. Natl. Acad. Sci. USA **86:**6935–6939.
2. **Amer, A. O., and M. S. Swanson.** 2005. Autophagy is an immediate macrophage response to *Legionella pneumophila*. Cell. Microbiol. **7:**765–778.
3. **Andrade, L. O., and N. W. Andrews.** 2004. Lysosomal fusion is essential for the retention of *Trypanosoma cruzi* inside host cells. J. Exp. Med. **200:**1135–1143.
4. **Asai, T., D. K. Howe, K. Nakajima, T. Nozaki, T. Takeuchi, and L. D. Sibley.** 1998. *Neospora caninum*: tachyzoites express a potent type-I nucleoside triphosphate hydrolase. Exp. Parasitol. **90:**277–285.
5. **Asai, T., and T. Kim.** 1987. Possible regulation mechanism of potent nucleoside triphosphate hydrolase in *Toxoplasma gondii*. Zentralbl. Bakteriol. Mikrobiol. Hyg. A **264:**464–467.
6. **Asai, T., S. Miura, L. D. Sibley, H. Okabayashi, and T. Takeuchi.** 1995. Biochemical and molecular characterization of nucleoside triphosphate hydrolase isozymes from the parasitic protozoan *Toxoplasma gondii*. J. Biol. Chem. **270:**11391–11397.
7. **Asai, T., W. J. O'Sullivan, and M. Tatibana.** 1983. A potent nucleoside triphosphate hydrolase from the parasitic protozoan *Toxoplasma gondii*. Purification, some properties, and activation by thiol compounds. J. Biol. Chem. **258:**6816–6822.
8. **Atkinson, B., K. Dwyer, K. Enjyoji, and S. C. Robson.** 2006. Ecto-nucleotidases of the CD39/NTPDase family modulate platelet activation and thrombus formation: potential as therapeutic targets. Blood Cells Mol. Dis. **36:**217–222.
9. **Barat, C., G. Martin, A. R. Beaudoin, J. Sevigny, and M. J. Tremblay.** 2007. The nucleoside triphosphate diphosphohydrolase-1/CD39 is incorporated into human immunodeficiency type 1 particles, where it remains biologically active. J. Mol. Biol. **371:**269–282.
10. **Barros, F. S., L. F. De Menezes, A. A. Pinheiro, E. F. Silva, A. H. Lopes, W. De Souza, and J. R. Meyer-Fernandes.** 2000. Ectonucleotide diphosphohydrolase activities in *Entamoeba histolytica*. Arch. Biochem. Biophys. **375:**304–314.
11. **Bermudes, D., K. R. Peck, M. A. Afifi, C. J. Beckers, and K. A. Joiner.** 1994. Tandemly repeated genes encode nucleoside triphosphate hydrolase isoforms secreted into the parasitophorous vacuole of *Toxoplasma gondii*. J. Biol. Chem. **269:**29252–29260.
12. **Berninsone, P., J. J. Miret, and C. B. Hirschberg.** 1994. The Golgi guanosine diphosphatase is required for transport of GDP-mannose into the lumen of *Saccharomyces cerevisiae* Golgi vesicles. J. Biol. Chem. **269:**207–211.
13. **Berredo-Pinho, M., C. E. Peres-Sampaio, P. P. Chrispim, R. Belmont-Firpo, A. P. Lemos, A. Martiny, M. A. Vannier-Santos, and J. R. Meyer-Fernandes.** 2001. A Mg-dependent ecto-ATPase in *Leishmania amazonensis* and its possible role in adenosine acquisition and virulence. Arch. Biochem. Biophys. **391:**16–24.
14. **Bigonnesse, F., S. A. Levesque, F. Kukulski, J. Lecka, S. C. Robson, M. J. Fernandes, and J. Sevigny.** 2004. Cloning and characterization of mouse nucleoside triphosphate diphosphohydrolase-8. Biochemistry **43:**5511–5519.
15. **Bisaggio, D. F., C. E. Peres-Sampaio, J. R. Meyer-Fernandes, and T. Souto-Padron.** 2003. Ecto-ATPase activity on the surface of *Trypanosoma cruzi* and its possible role in the parasite-host cell interaction. Parasitol. Res. **91:**273–282.
16. **Borsellino, G., M. Kleinewietfeld, D. Di Mitri, A. Sternjak, A. Diamantini, R. Giometto, S. Hopner, D. Centonze, G. Bernardi, M. L. Dell'Acqua, P. M. Rossini, L. Battistini, O. Rotzschke, and K. Falk.** 2007. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood **110:**1225–1232.
17. **Bours, M. J., E. L. Swennen, F. Di Virgilio, B. N. Cronstein, and P. C. Dagnelie.** 2006. Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol. Ther. **112:**358–404.
18. **Bruggemann, H., C. Cazalet, and C. Buchrieser.** 2006. Adaptation of

*Legionella pneumophila* to the host environment: role of protein secretion, effectors and eukaryotic-like proteins. Curr. Opin. Microbiol. **9:**86–94.

19. **Buell, C. R., V. Joardar, M. Lindeberg, J. Selengut, I. T. Paulsen, M. L. Gwinn, R. J. Dodson, R. T. Deboy, A. S. Durkin, J. F. Kolonay, R. Madupu, S. Daugherty, L. Brinkac, M. J. Beanan, D. H. Haft, W. C. Nelson, T. Davidsen, N. Zafar, L. Zhou, J. Liu, Q. Yuan, H. Khouri, N. Fedorova, B. Tran, D. Russell, K. Berry, T. Utterback, S. E. Van Aken, T. V. Feldblyum, M. D'Ascenzo, W. L. Deng, A. R. Ramos, J. R. Alfano, S. Cartinhour, A. K. Chatterjee, T. P. Delaney, S. G. Lazarowitz, G. B. Martin, D. J. Schneider, X. Tang, C. L. Bender, O. White, C. M. Fraser, and A. Collmer.** 2003. The complete genome sequence of the Arabidopsis and tomato pathogen *Pseudomonas syringae* pv. tomato DC3000. Proc. Natl. Acad. Sci. USA **100:**10181–10186.
20. **Burnstock, G.** 2007. Purine and pyrimidine receptors. Cell Mol. Life Sci. **64:**1471–1483.
21. **Burnstock, G.** 1972. Purinergic nerves. Pharmacol. Rev. **24:**509–581.
22. **Carlton, J. M., R. P. Hirt, J. C. Silva, A. L. Delcher, M. Schatz, Q. Zhao, J. R. Wortman, S. L. Bidwell, U. C. Alsmark, S. Besteiro, T. Sicheritz-Ponten, C. J. Noel, J. B. Dacks, P. G. Foster, C. Simillion, Y. Van de Peer, D. Miranda-Saavedra, G. J. Barton, G. D. Westrop, S. Muller, D. Dessi, P. L. Fiori, Q. Ren, I. Paulsen, H. Zhang, F. D. Bastida-Corcuera, A. Simoes-Barbosa, M. T. Brown, R. D. Hayes, M. Mukherjee, C. Y. Okumura, R. Schneider, A. J. Smith, S. Vanacova, M. Villalvazo, B. J. Haas, M. Pertea, T. V. Feldblyum, T. R. Utterback, C. L. Shu, K. Osoegawa, P. J. de Jong, I. Hrdy, L. Horvathova, Z. Zubacova, P. Dolezal, S. B. Malik, J. M. Logsdon, Jr., K. Henze, A. Gupta, C. C. Wang, R. L. Dunne, J. A. Upcroft, P. Upcroft, O. White, S. L. Salzberg, P. Tang, C. H. Chiu, Y. S. Lee, T. M. Embley, G. H. Coombs, J. C. Mottram, J. Tachezy, C. M. Fraser-Liggett, and P. J. Johnson.** 2007. Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Science **315:**207–212.
23. **Castro, R., K. Scott, T. Jordan, B. Evans, J. Craig, E. L. Peters, and K. Swier.** 2006. The ultrastructure of the parasitophorous vacuole formed by *Leishmania major*. J. Parasitol. **92:**1162–1170.
24. **Cazalet, C., C. Rusniok, H. Bruggemann, N. Zidane, A. Magnier, L. Ma, M. Tichit, S. Jarraud, C. Bouchier, F. Vandenesch, F. Kunst, J. Etienne, P. Glaser, and C. Buchrieser.** 2004. Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. Nat. Genet. **36:**1165–1173.
25. **Chen, J., K. S. de Felipe, M. Clarke, H. Lu, O. R. Anderson, G. Segal, and H. A. Shuman.** 2004. *Legionella* effectors that promote nonlytic release from protozoa. Science **303:**1358–1361.
26. **Chien, M., I. Morozova, S. Shi, H. Sheng, J. Chen, S. M. Gomez, G. Asamani, K. Hill, J. Nuara, M. Feder, J. Rineer, J. J. Greenberg, V. Steshenko, S. H. Park, B. Zhao, E. Teplitskaya, J. R. Edwards, S. Pampou, A. Georghiou, I. C. Chou, W. Iannuccilli, M. E. Ulz, D. H. Kim, A. Geringer-Sameth, C. Goldsberry, P. Morozov, S. G. Fischer, G. Segal, X. Qu, A. Rzhetsky, P. Zhang, E. Cayanis, P. J. De Jong, J. Ju, S. Kalachikov, H. A. Shuman, and J. J. Russo.** 2004. The genomic sequence of the accidental pathogen *Legionella pneumophila*. Science **305:**1966–1968.
27. **Chopra, P., A. Singh, A. Koul, S. Ramachandran, K. Drlica, A. K. Tyagi, and Y. Singh.** 2003. Cytotoxic activity of nucleoside diphosphate kinase secreted from *Mycobacterium tuberculosis*. Eur. J. Biochem. **270:**625–634.
28. **Cohn, C. S., and M. Gottlieb.** 1997. The acquisition of purines by trypanosomatids. Parasitol. Today **13:**231–235.
29. **Collopy-Junior, I., L. F. Kneipp, F. C. da Silva, M. L. Rodrigues, C. S. Alviano, and J. R. Meyer-Fernandes.** 2006. Characterization of an ecto-ATPase activity in *Fonsecaea pedrosoi*. Arch. Microbiol. **185:**355–362.
30. Reference deleted.
31. Reference deleted.
32. **Coppens, I., M. Andries, J. L. Liu, and M. F. Cesbron-Delauw.** 1999. Intracellular trafficking of dense granule proteins in *Toxoplasma gondii* and experimental evidences for a regulated exocytosis. Eur. J. Cell Biol. **78:**463–472.
33. **Coppens, I., J. D. Dunn, J. D. Romano, M. Pypaert, H. Zhang, J. C. Boothroyd, and K. A. Joiner.** 2006. *Toxoplasma gondii* sequesters lysosomes from mammalian hosts in the vacuolar space. Cell **125:**261–274.
34. **Costa-Ramos, C., and A. F. Rowley.** 2004. Effect of extracellular products of *Pseudoalteromonas atlantica* on the edible crab *Cancer pagurus*. Appl. Environ. Microbiol. **70:**729–735.
35. **Coutinho-Silva, R., J. L. Perfettini, P. M. Persechini, A. Dautry-Varsat, and D. M. Ojcius.** 2001. Modulation of P2Z/P2X(7) receptor activity in macrophages infected with *Chlamydia psittaci*. Am. J. Physiol. Cell Physiol. **280:** C81–C89.
36. **Coutinho-Silva, R., L. Stahl, M. N. Raymond, T. Jungas, P. Verbeke, G. Burnstock, T. Darville, and D. M. Ojcius.** 2003. Inhibition of chlamydial infectious activity due to P2X7R-dependent phospholipase D activation. Immunity **19:**403–412.
37. **Cox, M. A., B. Gomes, K. Palmer, K. Du, M. Wiekowski, B. Wilburn, M. Petro, C. C. Chou, C. Desquitado, M. Schwarz, C. Lunn, D. Lundell, S. K. Narula, P. J. Zavodny, and C. H. Jenh.** 2005. The pyrimidinergic P2Y6 receptor mediates a novel release of proinflammatory cytokines and chemokines in monocytic cells stimulated with UDP. Biochem. Biophys. Res. Commun. **330:**467–473.
38. **Cunha, B. A.** 2006. The atypical pneumonias: clinical diagnosis and importance. Clin. Microbiol. Infect. **12**(Suppl. 3)**:**12–24.
39. **Deaglio, S., K. M. Dwyer, W. Gao, D. Friedman, A. Usheva, A. Erat, J. F. Chen, K. Enjyoji, J. Linden, M. Oukka, V. K. Kuchroo, T. B. Strom, and S. C. Robson.** 2007. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J. Exp. Med. **204:**1257–1265.
40. **de Aguiar Matos, J. A., F. P. Borges, T. Tasca, M. R. Bogo, G. A. De Carli, M. da Graca Fauth, R. D. Dias, and C. D. Bonan.** 2001. Characterisation of an ATP diphosphohydrolase (apyrase, EC 3.6.1.5) activity in *Trichomonas vaginalis*. Int. J. Parasitol. **31:**770–775.
41. **de Felipe, K. S., S. Pampou, O. S. Jovanovic, C. D. Pericone, S. F. Ye, S. Kalachikov, and H. A. Shuman.** 2005. Evidence for acquisition of *Legionella* type IV secretion substrates via interdomain horizontal gene transfer. J. Bacteriol. **187:**7716–7726.
42. **de Jesus, J. B., A. A. de Sa Pinheiro, A. H. Lopes, and J. R. Meyer-Fernandes.** 2002. An ectonucleotide ATP-diphosphohydrolase activity in *Trichomonas vaginalis* stimulated by galactose and its possible role in virulence. Z Naturforsch. C **57:**890–896.
43. **De Jesus, J. B., M. A. Ferreira, P. Cuervo, C. Britto, F. C. e Silva-Filho, and J. R. Meyer-Fernandes.** 2006. Iron modulates ecto-phosphohydrolase activities in pathogenic trichomonads. Parasitol. Int. **55:**285–290.
44. **de Koning, H. P., D. J. Bridges, and R. J. Burchmore.** 2005. Purine and pyrimidine transport in pathogenic protozoa: from biology to therapy. FEMS Microbiol. Rev. **29:**987–1020.
45. **DeMarco, R., A. T. Kowaltowski, R. A. Mortara, and S. Verjovski-Almeida.** 2003. Molecular characterization and immunolocalization of *Schistosoma mansoni* ATP-diphosphohydrolase. Biochem. Biophys. Res. Commun. **307:** 831–838.
46. **de Sa Pinheiro, A. A., D. Cosentino-Gomes, A. Lanfredi-Rangel, R. B. Ferraro, W. De Souza, and J. R. Meyer-Fernandes.** 2008. *Giardia lamblia*: biochemical characterization of an ecto-ATPase activity. Exp. Parasitol. **119:**279–284.
47. **de Souza Leite, M., R. Thomaz, F. V. Fonseca, R. Panizzutti, A. E. Vercesi, and J. R. Meyer-Fernandes.** 2007. *Trypanosoma brucei brucei*: Biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. Exp. Parasitol. **115:**315–323.
48. **Douillet, C. D., W. P. Robinson, 3rd, P. M. Milano, R. C. Boucher, and P. B. Rich.** 2006. Nucleotides induce IL-6 release from human airway epithelia via P2Y2 and p38 MAPK-dependent pathways. Am. J. Physiol. Lung Cell Mol. Physiol. **291:**L734–L746.
49. **Drosopoulos, J. H.** 2002. Roles of Asp54 and Asp213 in Ca2+ utilization by soluble human CD39/ecto-nucleotidase. Arch. Biochem. Biophys. **406:**85–95.
50. **Drosopoulos, J. H., M. J. Broekman, N. Islam, C. R. Maliszewski, R. B. Gayle III, and A. J. Marcus.** 2000. Site-directed mutagenesis of human endothelial cell ecto-ADPase/soluble CD39: requirement of glutamate 174 and serine 218 for enzyme activity and inhibition of platelet recruitment. Biochemistry **39:**6936–6943.
51. **Enjyoji, K., J. Sevigny, Y. Lin, P. S. Frenette, P. D. Christie, J. S. Esch, 2nd, M. Imai, J. M. Edelberg, H. Rayburn, M. Lech, D. L. Beeler, E. Csizmadia, D. D. Wagner, S. C. Robson, and R. D. Rosenberg.** 1999. Targeted disruption of CD39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. Nat. Med. **5:**1010–1017.
52. **Fairbairn, I. P., C. B. Stober, D. S. Kumararatne, and D. A. Lammas.** 2001. ATP-mediated killing of intracellular mycobacteria by macrophages is a P2X(7)-dependent process inducing bacterial death by phagosome-lysosome fusion. J. Immunol. **167:**3300–3307.
53. **Faria-Pinto, P., M. N. Meirelles, H. L. Lenzi, E. M. Mota, M. L. Penido, P. M. Coelho, and E. G. Vasconcelos.** 2004. ATP diphosphohydrolase from *Schistosoma mansoni* egg: characterization and immunocytochemical localization of a new antigen. Parasitology **129:**51–57.
54. **Fernando, S. L., B. M. Saunders, R. Sluyter, K. K. Skarratt, H. Goldberg, G. B. Marks, J. S. Wiley, and W. J. Britton.** 2007. A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. Am. J. Respir. Crit. Care Med. **175:**360–366.
55. **Ferrari, D., P. Chiozzi, S. Falzoni, M. Dal Susino, L. Melchiorri, O. R. Baricordi, and F. Di Virgilio.** 1997. Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J. Immunol. **159:**1451–1458.
56. **Fields, B. S.** 1996. The molecular ecology of legionellae. Trends Microbiol. **4:**286–290.
57. **Fietto, J. L., R. DeMarco, I. P. Nascimento, I. M. Castro, T. M. Carvalho, W. de Souza, M. T. Bahia, M. J. Alves, and S. Verjovski-Almeida.** 2004. Characterization and immunolocalization of an NTP diphosphohydrolase of *Trypanosoma cruzi*. Biochem. Biophys. Res. Commun. **316:**454–460.
58. **Filippini, A., R. E. Taffs, T. Agui, and M. V. Sitkovsky.** 1990. Ecto-ATPase activity in cytolytic T-lymphocytes. Protection from the cytolytic effects of extracellular ATP. J. Biol. Chem. **265:**334–340.
59. **Fonseca, F. V., A. L. Fonseca de Souza, A. C. Mariano, P. F. Entringer, K. C. Gondim, and J. R. Meyer-Fernandes.** 2006. *Trypanosoma rangeli*:

characterization of a Mg-dependent ecto ATP-diphosphohydrolase activity. Exp. Parasitol. **112:**76–84.
60. **Fredholm, B. B.** 2007. Adenosine, an endogenous distress signal, modulates tissue damage and repair. Cell Death Differ. **14:**1315–1323.
61. **Gachet, C.** 2006. Regulation of platelet functions by P2 receptors. Annu. Rev. Pharmacol. Toxicol. **46:**277–300.
62. **Galka, F., S. Wai, H. Kusch, S. Engelmann, M. Hecker, B. Schmeck, S. Hippenstiel, B. Uhlin, and M. Steinert.** 2008. Proteomic characterization of the whole secretome of *Legionella pneumophila* and functional analysis of outer membrane vesicles. Infect. Immun. **76:**1825–1836.
63. **Gao, X. D., V. Kaigorodov, and Y. Jigami.** 1999. YND1, a homologue of GDA1, encodes membrane-bound apyrase required for Golgi N- and O-glycosylation in *Saccharomyces cerevisiae*. J. Biol. Chem. **274:**21450–21456.
64. **Gardner, M. J., N. Hall, E. Fung, O. White, M. Berriman, R. W. Hyman, J. M. Carlton, A. Pain, K. E. Nelson, S. Bowman, I. T. Paulsen, K. James, J. A. Eisen, K. Rutherford, S. L. Salzberg, A. Craig, S. Kyes, M. S. Chan, V. Nene, S. J. Shallom, B. Suh, J. Peterson, S. Angiuoli, M. Pertea, J. Allen, J. Selengut, D. Haft, M. W. Mather, A. B. Vaidya, D. M. Martin, A. H. Fairlamb, M. J. Fraunholz, D. S. Roos, S. A. Ralph, G. I. McFadden, L. M. Cummings, G. M. Subramanian, C. Mungall, J. C. Venter, D. J. Carucci, S. L. Hoffman, C. Newbold, R. W. Davis, C. M. Fraser, and B. Barrell.** 2002. Genome sequence of the human malaria parasite *Plasmodium falciparum*. Nature **419:**498–511.
65. **Gayle, R. B., 3rd, C. R. Maliszewski, S. D. Gimpel, M. A. Schoenborn, R. G. Caspary, C. Richards, K. Brasel, V. Price, J. H. Drosopoulos, N. Islam, T. N. Alyonycheva, M. J. Broekman, and A. J. Marcus.** 1998. Inhibition of platelet function by recombinant soluble ecto-ADPase/CD39. J. Clin. Investig. **101:**1851–1859.
66. **Gherardi, A., and M. E. Sarciron.** 2007. Molecules targeting the purine salvage pathway in Apicomplexan parasites. Trends Parasitol. **23:**384–389.
67. **Grinthal, A., and G. Guidotti.** 2004. Dynamic motions of CD39 transmembrane domains regulate and are regulated by the enzymatic active site. Biochemistry **43:**13849–13858.
68. **Grinthal, A., and G. Guidotti.** 2000. Substitution of His59 converts CD39 apyrase into an ADPase in a quaternary structure dependent manner. Biochemistry **39:**9–16.
69. **Handa, M., and G. Guidotti.** 1996. Purification and cloning of a soluble ATP-diphosphohydrolase (apyrase) from potato tubers (*Solanum tuberosum*). Biochem. Biophys. Res. Commun. **218:**916–923.
70. **Heine, P., N. Braun, J. Sevigny, S. C. Robson, J. Servos, and H. Zimmermann.** 2001. The C-terminal cysteine-rich region dictates specific catalytic properties in chimeras of the ectonucleotidases NTPDase1 and NTPDase2. Eur. J. Biochem. **268:**364–373.
71. **Herwaldt, B. L.** 1999. Leishmaniasis. Lancet **354:**1191–1199.
72. **Hicks-Berger, C. A., B. P. Chadwick, A. M. Frischauf, and T. L. Kirley.** 2000. Expression and characterization of soluble and membrane-bound human nucleoside triphosphate diphosphohydrolase 6 (CD39L2). J. Biol. Chem. **275:**34041–34045.
73. **Hopfe, M., and B. Henrich.** 2008. OppA, the ecto-ATPase of *Mycoplasma hominis* induces ATP release and cell death in HeLa cells. BMC Microbiol. **8:**55.
74. **Hopfe, M., and B. Henrich.** 2004. OppA, the substrate-binding subunit of the oligopeptide permease, is the major ecto-ATPase of *Mycoplasma hominis*. J. Bacteriol. **186:**1021–1928.
75. **Horwitz, M. A.** 1983. Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. J. Exp. Med. **158:**1319–1331.
76. **Horwitz, M. A.** 1983. The Legionnaires' disease bacterium (*Legionella pneumophila*) inhibits phagosome-lysosome fusion in human monocytes. J. Exp. Med. **158:**2108–2126.
77. **Imai, M., C. Goepfert, E. Kaczmarek, and S. C. Robson.** 2000. CD39 modulates IL-1 release from activated endothelial cells. Biochem. Biophys. Res. Commun. **270:**272–278.
78. **Ivens, A. C., C. S. Peacock, E. A. Worthey, L. Murphy, G. Aggarwal, M. Berriman, E. Sisk, M. A. Rajandream, E. Adlem, R. Aert, A. Anupama, Z. Apostolou, P. Attipoe, N. Bason, C. Bauser, A. Beck, S. M. Beverley, G. Bianchettin, K. Borzym, G. Bothe, C. V. Bruschi, M. Collins, E. Cadag, L. Ciarloni, C. Clayton, R. M. Coulson, A. Cronin, A. K. Cruz, R. M. Davies, J. De Gaudenzi, D. E. Dobson, A. Duesterhoeft, G. Fazelina, N. Fosker, A. C. Frasch, A. Fraser, M. Fuchs, C. Gabel, A. Goble, A. Goffeau, D. Harris, C. Hertz-Fowler, H. Hilbert, D. Horn, Y. Huang, S. Klages, A. Knights, M. Kube, N. Larke, L. Litvin, A. Lord, T. Louie, M. Marra, D. Masuy, K. Matthews, S. Michaeli, J. C. Mottram, S. Muller-Auer, H. Munden, S. Nelson, H. Norbertczak, K. Oliver, S. O'Neil, M. Pentony, T. M. Pohl, C. Price, B. Purnelle, M. A. Quail, E. Rabbinowitsch, R. Reinhardt, M. Rieger, J. Rinta, J. Robben, L. Robertson, J. C. Ruiz, S. Rutter, D. Saunders, M. Schafer, J. Schein, D. C. Schwartz, K. Seeger, A. Seyler, S. Sharp, H. Shin, D. Sivam, R. Squares, S. Squares, V. Tosato, C. Vogt, G. Volckaert, R. Wambutt, T. Warren, H. Wedler, J. Woodward, S. Zhou, W. Zimmermann, D. F. Smith, J. M. Blackwell, K. D. Stuart, B. Barrell, et al.** 2005. The genome of the kinetoplastid parasite, *Leishmania major*. Science **309:**436–442.
79. Reference deleted.
80. **Jesus, J. B., A. H. Lopes, and J. R. Meyer-Fernandes.** 2002. Characterization of an ecto-ATPase of *Tritrichomonas foetus*. Vet. Parasitol. **103:**29–42.
81. **Johnson, M. S., K. W. Broady, and A. M. Johnson.** 1999. Differential recognition of *Toxoplasma gondii* recombinant nucleoside triphosphate hydrolase isoforms by naturally infected human sera. Int. J. Parasitol. **29:**1893–1905.
82. **Junior, I. C., M. L. Rodrigues, C. S. Alviano, L. R. Travassos, and J. R. Meyer-Fernandes.** 2005. Characterization of an ecto-ATPase activity in *Cryptococcus neoformans*. FEMS Yeast Res. **5:**899–907.
83. **Kaczmarek, E., K. Koziak, J. Sevigny, J. B. Siegel, J. Anrather, A. R. Beaudoin, F. H. Bach, and S. C. Robson.** 1996. Identification and characterization of CD39/vascular ATP diphosphohydrolase. J. Biol. Chem. **271:**33116–33122.
84. **Kikuchi, T., T. Furuta, and S. Kojima.** 2001. Membrane localization and demonstration of isoforms of nucleoside triphosphate hydrolase from *Toxoplasma gondii*. Parasitology **122:**15–23.
85. **Kikuchi, T., T. Kobayashi, K. Gomi, T. Suzuki, Y. Tokue, A. Watanabe, and T. Nukiwa.** 2004. Dendritic cells pulsed with live and dead *Legionella pneumophila* elicit distinct immune responses. J. Immunol. **172:**1727–1734.
86. **Kirchhoff, L. V., A. A. Gam, and F. C. Gilliam.** 1987. American trypanosomiasis (Chagas' disease) in Central American immigrants. Am. J. Med. **82:**915–920.
87. **Kirley, T. L., F. Yang, and V. V. Ivanenkov.** 2001. Site-directed mutagenesis of human nucleoside triphosphate diphosphohydrolase 3: the importance of conserved glycine residues and the identification of additional conserved protein motifs in eNTPDases. Arch. Biochem. Biophys. **395:**94–102.
88. **Knowles, A. F., and C. Li.** 2006. Molecular cloning and characterization of expressed human ecto-nucleoside triphosphate diphosphohydrolase 8 (E-NTPDase 8) and its soluble extracellular domain. Biochemistry **45:**7323–7333.
89. **Kobayashi, D., S. Ohkubo, and N. Nakahata.** 2006. Contribution of extracellular signal-regulated kinase to UTP-induced interleukin-6 biosynthesis in HaCaT keratinocytes. J. Pharmacol. Sci. **102:**368–376.
90. **Kukulski, F., F. Ben Yebdri, J. Lefebvre, M. Warny, P. A. Tessier, and J. Sevigny.** 2007. Extracellular nucleotides mediate LPS-induced neutrophil migration in vitro and in vivo. J. Leukoc. Biol. **81:**1269–1275.
91. **Kukulski, F., S. A. Levesque, E. G. Lavoie, J. Lecka, F. Bigonnesse, A. F. Knowles, S. C. Robson, T. L. Kirley, and J. Sevigny.** 2005. Comparative hydrolysis of P2 receptor agonists by NTPDases 1, 2, 3 and 8. Purinergic Signalling **1:**193–204.
92. **Kusner, D. J., and J. A. Barton.** 2001. ATP stimulates human macrophages to kill intracellular virulent *Mycobacterium tuberculosis* via calcium-dependent phagosome-lysosome fusion. J. Immunol. **167:**3308–3315.
93. **Langston, H. P., Y. Ke, A. T. Gewirtz, K. E. Dombrowski, and J. A. Kapp.** 2003. Secretion of IL-2 and IFN-gamma, but not IL-4, by antigen-specific T cells requires extracellular ATP. J. Immunol. **170:**2962–2970.
94. **Leal, D. B., C. A. Streher, M. Bertoncheli Cde, L. F. Carli, C. A. Leal, J. E. da Silva, V. M. Morsch, and M. R. Schetinger.** 2005. HIV infection is associated with increased NTPDase activity that correlates with CD39-positive lymphocytes. Biochim. Biophys. Acta **1746:**129–134.
95. **Letunic, I., R. R. Copley, B. Pils, S. Pinkert, J. Schultz, and P. Bork.** 2006. SMART 5: domains in the context of genomes and networks. Nucleic Acids Res. **34:**D257–D260.
96. **Levano-Garcia, J., R. A. Mortara, S. Verjovski-Almeida, and R. DeMarco.** 2007. Characterization of *Schistosoma mansoni* ATPDase2 gene, a novel apyrase family member. Biochem. Biophys. Res. Commun. **352:**384–389.
97. **Luiz Oliveira Penido, M., D. M. Resende, M. A. Vianello, F. Humberto da Silveira Bordin, A. A. Jacinto, W. D. Dias, M. A. Montesano, D. L. Nelson, P. Marcos Zech Coelho, and E. G. Vasconcelos.** 2007. A new series of schistosomicide drugs, the alkylaminoalkanethiosulfuric acids, partially inhibit the activity of *Schistosoma mansoni* ATP diphosphohydrolase. Eur. J. Pharmacol. **570:**10–17.
98. **Maioli, T. U., E. Takane, R. M. Arantes, J. L. Fietto, and L. C. Afonso.** 2004. Immune response induced by New World *Leishmania* species in C57BL/6 mice. Parasitol. Res. **94:**207–212.
99. **Marcus, A. J., M. J. Broekman, J. H. Drosopoulos, N. Islam, T. N. Alyonycheva, L. B. Safier, K. A. Hajjar, D. N. Posnett, M. A. Schoenborn, K. A. Schooley, R. B. Gayle, and C. R. Maliszewski.** 1997. The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39. J. Clin. Investig. **99:**1351–1360.
100. **Matthews, K. R.** 2005. The developmental cell biology of *Trypanosoma brucei*. J. Cell Sci. **118:**283–290.
101. **May, R. C., and L. M. Machesky.** 2001. Phagocytosis and the actin cytoskeleton. J. Cell Sci. **114:**1061–1077.
102. **McKee, A. S., F. Dzierszinski, M. Boes, D. S. Roos, and E. J. Pearce.** 2004. Functional inactivation of immature dendritic cells by the intracellular parasite *Toxoplasma gondii*. J. Immunol. **173:**2632–2640.
103. **Meyer-Fernandes, J. R., P. M. Dutra, C. O. Rodrigues, J. Saad-Nehme, and A. H. Lopes.** 1997. Mg-dependent ecto-ATPase activity in *Leishmania tropica*. Arch. Biochem. Biophys. **341:**40–46.
104. **Meyer-Fernandes, J. R., J. Saad-Nehme, C. E. Peres-Sampaio, R. Belmont-**

**Firpo, D. F. Bisaggio, L. C. Do Couto, A. L. Fonseca De Souza, A. H. Lopes, and T. Souto-Padron.** 2004. A Mg-dependent ecto-ATPase is increased in the infective stages of *Trypanosoma cruzi*. Parasitol. Res. **93:**41–50.

105. **Mizumoto, N., T. Kumamoto, S. C. Robson, J. Sevigny, H. Matsue, K. Enjyoji, and A. Takashima.** 2002. CD39 is the dominant Langerhans cell-associated ecto-NTPDase: modulatory roles in inflammation and immune responsiveness. Nat. Med. **8:**358–365.
106. **Munagala, N. R., and C. C. Wang.** 2003. Adenosine is the primary precursor of all purine nucleotides in *Trichomonas vaginalis*. Mol. Biochem. Parasitol. **127:**143–149.
107. **Nakaar, V., C. J. Beckers, V. Polotsky, and K. A. Joiner.** 1998. Basis for substrate specificity of the *Toxoplasma gondii* nucleoside triphosphate hydrolase. Mol. Biochem. Parasitol. **97:**209–220.
108. **Nakaar, V., D. Bermudes, K. R. Peck, and K. A. Joiner.** 1998. Upstream elements required for expression of nucleoside triphosphate hydrolase genes of *Toxoplasma gondii*. Mol. Biochem. Parasitol. **92:**229–239.
109. **Nakaar, V., B. U. Samuel, E. O. Ngo, and K. A. Joiner.** 1999. Targeted reduction of nucleoside triphosphate hydrolase by antisense RNA inhibits *Toxoplasma gondii* proliferation. J. Biol. Chem. **274:**5083–5087.
110. **Ngo, H. M., E. O. Ngo, D. J. Bzik, and K. A. Joiner.** 2000. *Toxoplasma gondii*: are host cell adenosine nucleotides a direct source for purine salvage? Exp. Parasitol. **95:**148–153.
111. **Okenu, D. M., K. N. Opara, R. I. Nwuba, and M. Nwagwu.** 1999. Purification and characterisation of an extracellularly released protease of *Trypanosoma brucei*. Parasitol. Res. **85:**424–428.
112. **Osim, E. E., B. J. Adegunloye, and A. O. Emeribe.** 1991. In vivo platelet aggregation in acute malaria. Acta Trop. **49:**227–232.
113. **Papanikolaou, A., A. Papafotika, C. Murphy, T. Papamarcaki, O. Tsolas, M. Drab, T. V. Kurzchalia, M. Kasper, and S. Christoforidis.** 2005. Cholesterol-dependent lipid assemblies regulate the activity of the ecto-nucleotidase CD39. J. Biol. Chem. **280:**26406–26414.
114. **Peacock, C. S., K. Seeger, D. Harris, L. Murphy, J. C. Ruiz, M. A. Quail, N. Peters, E. Adlem, A. Tivey, M. Aslett, A. Kerhornou, A. Ivens, A. Fraser, M. A. Rajandream, T. Carver, H. Norbertczak, T. Chillingworth, Z. Hance, K. Jagels, S. Moule, D. Ormond, S. Rutter, R. Squares, S. Whitehead, E. Rabbinowitsch, C. Arrowsmith, B. White, S. Thurston, F. Bringaud, S. L. Baldauf, A. Faulconbridge, D. Jeffares, D. P. Depledge, S. O. Oyola, J. D. Hilley, L. O. Brito, L. R. Tosi, B. Barrell, A. K. Cruz, J. C. Mottram, D. F. Smith, and M. Berriman.** 2007. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. Nat. Genet. **39:**839–847.
115. **Peres-Sampaio, C. E., E. E. de Almeida-Amaral, N. L. Giarola, and J. R. Meyer-Fernandes.** 2008. *Leishmania amazonensis*: effects of heat shock on ecto-ATPase activity. Exp. Parasitol. **119:**135–143.
116. **Peres-Sampaio, C. E., S. T. Palumbo, and J. R. Meyer-Fernandes.** 2001. An ecto-ATPase activity present in *Leishmania tropica* stimulated by dextran sulfate. Z. Naturforsch. C **56:**820–825.
117. **Pine, L., M. J. Franzus, and G. B. Malcolm.** 1986. Guanine is a growth factor for *Legionella* species. J. Clin. Microbiol. **23:**163–169.
118. **Pinheiro, C. M., E. S. Martins-Duarte, R. B. Ferraro, A. L. Fonseca de Souza, M. T. Gomes, A. H. Lopes, M. A. Vannier-Santos, A. L. Santos, and J. R. Meyer-Fernandes.** 2006. *Leishmania amazonensis*: biological and biochemical characterization of ecto-nucleoside triphosphate diphosphohydrolase activities. Exp. Parasitol. **114:**16–25.
119. **Pizzirani, C., D. Ferrari, P. Chiozzi, E. Adinolfi, D. Sandona, E. Savaglio, and F. Di Virgilio.** 2007. Stimulation of P2 receptors causes release of IL-1β-loaded microvesicles from human dendritic cells. Blood **109:**3856–3864.
120. **Polla, B. S.** 1991. Heat shock proteins in host-parasite interactions. Immunol. Today **12:**A38–A41.
121. **Robson, S. C., J. Sevigny, and H. Zimmermann.** 2006. The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance. Purinergic Signalling **2:**409–430.
122. **Sacks, D., and A. Sher.** 2002. Evasion of innate immunity by parasitic protozoa. Nat. Immunol. **3:**1041–1047.
123. **Sansom, F. M., H. J. Newton, S. Crikis, N. P. Cianciotto, P. J. Cowan, A. J. d'Apice, and E. L. Hartland.** 2007. A bacterial ecto-triphosphate diphosphohydrolase similar to human CD39 is essential for intracellular multiplication of *Legionella pneumophila*. Cell. Microbiol. **9:**1922–1935.
124. **Sansom, F. M., P. Riedmaier, H. J. Newton, M. A. Dunstone, C. E. Muller, H. Stephan, E. Byres, T. Beddoe, J. Rossjohn, P. J. Cowan, A. J. d'Apice, S. C. Robson, and E. L. Hartland.** 2008. Enzymatic properties of an ecto-nucleoside triphosphate diphosphohydrolase from *Legionella pneumophila*: substrate specificity and requirement for virulence. J. Biol. Chem. **283:**12909–12918.
125. **Schnurr, M., F. Then, P. Galambos, C. Scholz, B. Siegmund, S. Endres, and A. Eigler.** 2000. Extracellular ATP and TNF-alpha synergize in the activation and maturation of human dendritic cells. J. Immunol. **165:**4704–4709.
126. **Schulte am Esch, J., II, J. Sevigny, E. Kaczmarek, J. B. Siegel, M. Imai, K. Koziak, A. R. Beaudoin, and S. C. Robson.** 1999. Structural elements and limited proteolysis of CD39 influence ATP diphosphohydrolase activity. Biochemistry **38:**2248–2258.
127. **Schultz, J., F. Milpetz, P. Bork, and C. P. Ponting.** 1998. SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Natl. Acad. Sci. USA **95:**5857–5864.
128. **Schwebke, J. R., and D. Burgess.** 2004. Trichomoniasis. Clin. Microbiol. Rev. **17:**794–803.
129. **Shi, J. D., T. Kukar, C. Y. Wang, Q. Z. Li, P. E. Cruz, A. Davoodi-Semiromi, P. Yang, Y. Gu, W. Lian, D. H. Wu, and J. X. She.** 2001. Molecular cloning and characterization of a novel mammalian endo-apyrase (LALP1). J. Biol. Chem. **276:**17474–17478.
130. **Sibley, L. D., I. R. Niesman, T. Asai, and T. Takeuchi.** 1994. *Toxoplasma gondii*: secretion of a potent nucleoside triphosphate hydrolase into the parasitophorous vacuole. Exp. Parasitol. **79:**301–311.
131. **Silverman, J. A., H. Qi, A. Riehl, C. Beckers, V. Nakaar, and K. A. Joiner.** 1998. Induced activation of the *Toxoplasma gondii* nucleoside triphosphate hydrolase leads to depletion of host cell ATP levels and rapid exit of intracellular parasites from infected cells. J. Biol. Chem. **273:**12352–12359.
132. **Simpson, A. J., M. D. Schryer, I. M. Cesari, W. H. Evans, and S. R. Smithers.** 1981. Isolation and partial characterization of the tegumental outer membrane of adult *Schistosoma mansoni*. Parasitology **83:**163–177.
133. **Sissons, J., S. Alsam, S. Jayasekera, and N. A. Khan.** 2004. Ecto-ATPases of clinical and non-clinical isolates of *Acanthamoeba*. Microb. Pathog. **37:**231–239.
134. **Smith, T. M., and T. L. Kirley.** 1999. Site-directed mutagenesis of a human brain ecto-apyrase: evidence that the E-type ATPases are related to the actin/heat shock 70/sugar kinase superfamily. Biochemistry **38:**321–328.
135. **Smith, T. M., S. A. Lewis Carl, and T. L. Kirley.** 1999. Mutagenesis of two conserved tryptophan residues of the E-type ATPases: inactivation and conversion of an ecto-apyrase to an ecto-NTPase. Biochemistry **38:**5849–5857.
136. **Snoeijer, C. Q., G. F. Picchi, B. P. Dambros, M. Steindel, S. Goldenberg, S. P. Fragoso, D. M. Lorenzini, and E. C. Grisard.** 2004. Trypanosoma rangeli transcriptome project: generation and analysis of expressed sequence tags. Kinetoplastid Biol. Dis. **3:**1.
137. **Sorensen, S. W., C. J. Billington, S. A. Norris, J. E. Briggs, M. T. Reding, and G. A. Filice.** 1997. *Toxoplasma gondii*: metabolism of intracellular tachyzoites is affected by host cell ATP production. Exp. Parasitol. **85:**101–104.
138. **Srichaikul, T., C. Pulket, T. Sirisatepisarn, and W. Prayoonwiwat.** 1988. Platelet dysfunction in malaria. Southeast Asian J. Trop. Med. Public Health **19:**225–233.
139. **Stanley, R. G., J. R. Ngaiza, E. Wambayi, J. Lewis, and M. J. Doenhoff.** 2003. Platelets as an innate defence mechanism against *Schistosoma mansoni* infections in mice. Parasite Immunol. **25:**467–473.
140. **Stommel, E. W., E. Cho, J. A. Steide, R. Seguin, A. Barchowsky, J. D. Schwartzman, and L. H. Kasper.** 2001. Identification and role of thiols in *Toxoplasma gondii* egress. Exp. Biol. Med. **226:**229–236.
141. **Stommel, E. W., K. H. Ely, J. D. Schwartzman, and L. H. Kasper.** 1997. *Toxoplasma gondii*: dithiol-induced Ca2+ flux causes egress of parasites from the parasitophorous vacuole. Exp. Parasitol. **87:**88–97.
142. **Stout, J. G., and T. L. Kirley.** 1996. Control of cell membrane ecto-ATPase by oligomerization state: intermolecular cross-linking modulates ATPase activity. Biochemistry **35:**8289–8298.
143. **Swanson, M. S., and B. K. Hammer.** 2000. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. Annu. Rev. Microbiol. **54:**567–613.
144. **Tanowitz, H. B., E. R. Burns, A. K. Sinha, N. N. Kahn, S. A. Morris, S. M. Factor, V. B. Hatcher, J. P. Bilezikian, S. G. Baum, and M. Wittner.** 1990. Enhanced platelet adherence and aggregation in Chagas' disease: a potential pathogenic mechanism for cardiomyopathy. Am. J. Trop. Med. Hyg. **43:**274–281.
145. **Tasca, T., C. D. Bonan, G. A. De Carli, and J. J. Sarkis.** 2004. *Trichomonas vaginalis*: cytochemical localization of a NTPDase1 and an ecto-5′-nucleotidase and effects of adenine nucleotides on cellular viability. Parasitol. Res. **93:**300–303.
146. **Tasca, T., C. D. Bonan, G. A. De Carli, J. J. Sarkis, and J. F. Alderete.** 2005. Heterogeneity in extracellular nucleotide hydrolysis among clinical isolates of *Trichomonas vaginalis*. Parasitology **131:**71–78.
147. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
148. **Torres, C. R., E. G. Vasconcelos, S. T. Ferreira, and S. Verjovski-Almeida.** 1998. Divalent cation dependence and inhibition of *Schistosoma mansoni* ATP diphosphohydrolase by fluorosulfonylbenzoyl adenosine. Eur. J. Biochem. **251:**516–521.
149. **Umekita, L. F., R. M. Piazza, and I. Mota.** 1994. Role of platelets and complement in the clearance of epimastigote forms of *Trypanosoma cruzi*. Braz. J. Med. Biol. Res. **27:**2391–2399.
150. **Van der Pol, B.** 2007. *Trichomonas vaginalis* infection: the most prevalent nonviral sexually transmitted infection receives the least public health attention. Clin. Infect. Dis. **44:**23–25.
151. **Vasconcelos, E. G., S. T. Ferreira, T. M. Carvalho, W. Souza, A. M. Kettlun,**

**M. Mancilla, M. A. Valenzuela, and S. Verjovski-Almeida.** 1996. Partial purification and immunohistochemical localization of ATP diphosphohydrolase from *Schistosoma mansoni*. Immunological cross-reactivities with potato apyrase and *Toxoplasma gondii* nucleoside triphosphate hydrolase. J. Biol. Chem. **271:**22139–22145.

152. **Vasconcelos, E. G., P. S. Nascimento, M. N. Meirelles, S. Verjovski-Almeida, and S. T. Ferreira.** 1993. Characterization and localization of an ATP-diphosphohydrolase on the external surface of the tegument of *Schistosoma mansoni*. Mol. Biochem. Parasitol. **58:**205–214.
153. **Wang, T. F., and G. Guidotti.** 1996. CD39 is an ecto-(Ca2+,Mg2+)-apyrase. J. Biol. Chem. **271:**9898–9901.
154. **Wang, T. F., and G. Guidotti.** 1998. Golgi localization and functional expression of human uridine diphosphatase. J. Biol. Chem. **273:**11392–11399.
155. **Wang, T. F., Y. Ou, and G. Guidotti.** 1998. The transmembrane domains of ectoapyrase (CD39) affect its enzymatic activity and quaternary structure. J. Biol. Chem. **273:**24814–24821.
156. **Warny, M., S. Aboudola, S. C. Robson, J. Sevigny, D. Communi, S. P. Soltoff, and C. P. Kelly.** 2001. P2Y(6) nucleotide receptor mediates monocyte interleukin-8 production in response to UDP or lipopolysaccharide. J. Biol. Chem. **276:**26051–26056.
157. **Woo, S. R., R. G. Barletta, and C. J. Czuprynski.** 2007. Extracellular ATP is cytotoxic to mononuclear phagocytes but does not induce killing of intracellular *Mycobacterium avium subsp. paratuberculosis*. Clin. Vaccine Immunol. **14:**1078–1083.
158. **Wu, Y., X. Sun, E. Kaczmarek, K. M. Dwyer, E. Bianchi, A. Usheva, and S. C. Robson.** 2006. RanBPM associates with CD39 and modulates ecto-nucleotidase activity. Biochem. J. **396:**23–30.
159. **Yanagisawa, K., D. Resnick, C. Abeijon, P. W. Robbins, and C. B. Hirschberg.** 1990. A guanosine diphosphatase enriched in Golgi vesicles of *Saccharomyces cerevisiae*. Purification and characterization. J. Biol. Chem. **265:**19351–19355.
160. **Yang, F., C. A. Hicks-Berger, T. M. Smith, and T. L. Kirley.** 2001. Site-directed mutagenesis of human nucleoside triphosphate diphosphohydrolase 3: the importance of residues in the apyrase conserved regions. Biochemistry **40:**3943–3950.
161. **Yeung, G., J. J. Mulero, D. W. McGowan, S. S. Bajwa, and J. E. Ford.** 2000. CD39L2, a gene encoding a human nucleoside diphosphatase, predominantly expressed in the heart. Biochemistry **39:**12916–12923.
162. **Yilmaz, O., L. Yao, K. Maeda, T. M. Rose, E. L. Lewis, M. Duman, R. J. Lamont, and D. M. Ojcius.** 2008. ATP scavenging by the intracellular pathogen *Porphyromonas gingivalis* inhibits P2X(7)-mediated host-cell apoptosis. Cell. Microbiol. **10:**863–875.
163. **Zaborina, O., X. Li, G. Cheng, V. Kapatral, and A. M. Chakrabarty.** 1999. Secretion of ATP-utilizing enzymes, nucleoside diphosphate kinase and ATPase, by *Mycobacterium bovis* BCG: sequestration of ATP from macrophage P2Z receptors? Mol. Microbiol. **31:**1333–1343.
164. **Zaborina, O., N. Misra, J. Kostal, S. Kamath, V. Kapatral, M. E. El-Idrissi, B. S. Prabhakar, and A. M. Chakrabarty.** 1999. P2Z-independent and P2Z receptor-mediated macrophage killing by *Pseudomonas aeruginosa* isolated from cystic fibrosis patients. Infect. Immun. **67:**5231–5242.
165. **Zaki, M. H., T. Akuta, and T. Akaike.** 2005. Nitric oxide-induced nitrative stress involved in microbial pathogenesis. J. Pharmacol. Sci. **98:**117–129.
166. **Zebisch, M., and N. Sträter.** 2008. Structural insight into signal conversion and inactivation by NTPDase2 in purinergic signaling Proc. Natl. Acad. Sci. USA **105:**6882–6887.
167. **Zhang, D., R. Y. Gaji, and D. K. Howe.** 2006. Identification of a dithiol-dependent nucleoside triphosphate hydrolase in *Sarcocystis neurona*. Int. J. Parasitol. **36:**1197–1204.
168. **Zimmermann, H.** 2001. Ectonucleotidases: some recent developments and a note on nomenclature. Drug Dev. Res. **52:**44–56.