The protozoan parasite *Leishmania* is responsible for a variety of clinical manifestations, ranging from mild cutaneous infections to life-threatening visceral diseases (12). Pentavalent antimony [Sb(V)] containing compounds such as sodium stibogluconate (Pentostam) and N-methylglucamine (Glucantime) remain the first-line drugs against all forms of *Leishmania* infections in developing countries (19), but their efficacies are threatened by resistant parasites in several regions where the disease is endemic (9, 17, 21). Our previous work on metal resistance in *Leishmania* (Glucantime) remain the first-line drugs against all forms of *Leishmania* infections in developing countries (19), but their efficacies are threatened by resistant parasites in several regions where the disease is endemic (9, 17, 21). Our previous work on metal resistance in *Leishmania* led to the definition of a model of resistance involving proteins of the ATP-binding cassette (ABC) superfamily (20). ABC proteins form one of the largest families of transmembrane proteins and are characterized by the presence of the strongly conserved nucleotide-binding domain (NBD), which is composed of three major motifs. Along with the Walker A and B motifs found in many nucleotide-binding proteins (23), the NBD is composed of a characteristic ABC signature “C” motif located just upstream of the Walker B site (13). Eukaryotic ABC proteins can be divided into eight different subfamilies (ABCA to ABCH) on the basis of gene structure and NBD sequence homologies. A previous survey indicated the presence of 42 ABC protein-coding genes in the genomes of *Leishmania major* and *Leishmania infantum* (16), but the latest version of the *L. infantum* genome (GeneDB V3.0) revealed the presence of a new member of the ABC superfamily (LinJ24_V3.1510). This gene seems to be specific to *L. infantum* since it is absent from the genome of *L. major* (GeneDB V5.1) and is found as a pseudogene of low homology in the genome of *Leishmania braziliensis* (GeneDB V2.0). A phylogenetic analysis of the *L. infantum* ABC proteins revealed that LinJ24_V3.1510 is the most divergent member of the ABC subfamily in *Leishmania* (results not shown) and has been named ABCC9. While the Walker A and B motifs are well conserved in the ABC proteins of *Leishmania*, some conserved residues essential to the function of ABC proteins (7) are absent from the C motif of ABCC9 (Fig. 1), and the functionality of this protein in *L. infantum* still needs to be established.

**Intracellular Localization of the ABCC Proteins of *Leishmania* and Their Role in Resistance to Antimonials**

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The ABCC subfamily of proteins is composed of nine members in *Leishmania*. We report that all of these proteins have an intracellular localization and that the overexpression of at least four members, ABCC3, ABCC4, ABCC5, and ABCC7, can confer resistance to antimonials, the first-line drug against *Leishmania*.

The involvement of the intracellular ABCC proteins MRPA/ABCC3 and PRP1/ABCC7 in antimony resistance has already
been reported (2, 3, 15). However, only MRPA/ABCC3 has been shown to confer resistance both to Sb(V) and to Sb(III) (5) and to be amplified in field isolates derived from patients unresponsive to antimonials (18). Since none of the ABCC protein was located in the plasma membrane (Fig. 2), we next tested if the ABCC1, ABCC2, ABCC4, ABCC5, ABCC6, ABCC8, and ABCC9 proteins could act as intracellular transporters associated with resistance to Sb(III), the biologically active form of antimony. Growth curve experiments showed that the previously described MRPA/ABCC3 and PRP1/ABCC7 proteins were the only ABCC proteins associated with Sb(III) resistance when overexpressed in a wild-type (WT) background of *L. infantum* (not shown) or *Leishmania tarentolae* (Table 1). However, previous studies have shown that MRPA gave higher resistance levels when its gene was transfected in the cell line *L. tarentolae* As20.3rev (8, 11), and we thus transfected the various ABCC constructs in this partial revertant line. The *L. tarentolae* As20.3rev cell line was generated from the Sb(III)-resistant mutant *L. tarentolae As20.3* by successive passages in the absence of antimony. The As20.3rev cell line is more susceptible than the parent mutant but remains considerably more resistant than its parental WT strain (Table 1). The overexpression of ABCC4-GFP and ABCC5-GFP in *L. tarentolae* As20.3rev resulted in a highly reproducible twofold increase in resistance to Sb(III) (Table 1). The ABCC4-GFP, ABCC5-GFP, and GFP-ABCC5 fusions were functional since similar resistance levels were observed with the unfused version of the proteins in *L. tarentolae* As20.3rev (Table 1). The other ABCC proteins were not associated with significant antimony resistance when overexpressed in *L. tarentolae* As20.3rev (apart from the previously described MRPA/ABCC3 and PRP1/ABCC7 proteins) (Table 1).

It has been reported that increased levels of cellular thiols are required for antimony resistance in *Leishmania*. Accordingly, the TSH levels are increased 10 times in the *L. tarentolae* As20.3 resistant mutant and remain at least threefold higher in the partial revertant line *L. tarentolae* As20.3rev than in the WT *L. tarentolae* (8, 10, 11). By analogy with the GS-X system and as previously demonstrated for MRPA/ABCC3 (15), it might be possible that the ABCC4 and ABCC5 proteins trans-

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**FIG. 1.** Sequence alignment of the NBDs of *L. infantum* ABCC proteins. Shown are N-terminal NBDs (A) and C-terminal NBDs (B). The Walker A, Walker B, and signature C motifs of both NBDs are shown as boxes. An ABCC-like motif found in most ABCC proteins is underlined. The alignment was performed by using ClustalW (22), and the figure was formatted using Jalview (1).
ABCC4 and ABCC5 being specific to the thiol levels or the formation of the conjugates would be the port Sb(III) as part of TSH complexes, where the increased parasites. Shown are Leishmania

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crease in TSH-transferase activity associated with Sb(III) re-

As20.3rev background, but in light of recent results, an in-

GFP images (right). are from differential interference contrast images (DIC1) (left), fluo-

GFP) and to the posterior end of the parasites (ABCC8-GFP). Shown along the longitudinal axis of the parasite (ABCC4-GFP and ABCC5-GFP, ABCC2-GFP, and ABCC6-GFP) to a tubular organelle oriented

monials in field isolates.

studies will be required to isolate this efflux system and to

ABCC proteins, given their intracellular localization. Further

plasma membrane of the parasite is probably unrelated to

that the antimony efflux system previously described at the

protein subfamily in antimony resistance in vitro and suggested

This study highlighted the role of the Leishmania ABC

port Sb(III) as part of TSH complexes, where the increased thiol levels or the formation of the conjugates would be the rate-limiting steps of the transport process. This could explain the resistance phenotype conferred by the overexpression of ABCC4 and ABCC5 being specific to the L. tarentolae As20.3rev background, but in light of recent results, an increase in TSH-transferase activity associated with Sb(III) resistance in Leishmania parasites is unlikely (24).

This study highlighted the role of the Leishmania ABC protein subfamily in antimy resistance in vitro and suggested that the antimony efflux system previously described at the plasma membrane of the parasite is probably unrelated to ABCC proteins, given their intracellular localization. Further studies will be required to isolate this efflux system and to further study the role of ABCC proteins in resistance to antimonials in field isolates.

FIG. 2. Intracellular localization of the entire ABCC subfamily in Leishmania parasites. Shown are L. infantum promastigotes expressing fluorescence signals to a network of intracellular membranes (ABCC1-GFP, ABCC2-GFP, and ABCC6-GFP) to a tubular organelle oriented along the longitudinal axis of the parasite (ABCC4-GFP and ABCC5-GFP) and to the posterior end of the parasites (ABCC8-GFP). Shown are from differential interference contrast images (DIC1) (left), fluorescence only images (GFP) (middle), and a merge of the DIC1 and GFP images (right).

TABLE 1. Antimy resistance in L. tarentolae transfectants

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Antimy resistance</th>
<th>EC50 (µM)b</th>
<th>Fold increaseb</th>
</tr>
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<tr>
<td>Tarll WT</td>
<td></td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>Tarll WT + ABCC1-GFP</td>
<td></td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>Tarll WT + ABCC2-GFP</td>
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<td>0.14</td>
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</tr>
<tr>
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<td>0.37</td>
<td>2.5</td>
</tr>
<tr>
<td>Tarll WT + ABCC4-GFP</td>
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<td>0.14</td>
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<tr>
<td>Tarll WT + ABCC5-GFP</td>
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<td>0.15</td>
<td>1</td>
</tr>
<tr>
<td>Tarll WT + ABCC7-GFP</td>
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<td>Tarll WT + ABCC8-GFP</td>
<td></td>
<td>0.14</td>
<td>1</td>
</tr>
<tr>
<td>Tarll WT + liABCC9</td>
<td></td>
<td>0.15</td>
<td>1</td>
</tr>
<tr>
<td>Tarll As20.3rev</td>
<td></td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Tarll As20.3rev + ABCC1-GFP</td>
<td></td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Tarll As20.3rev + ABCC2-GFP</td>
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<tr>
<td>Tarll As20.3rev + ABCC3/MRPA</td>
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<tr>
<td>Tarll As20.3rev + liABCC9</td>
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</tr>
</tbody>
</table>

a Average of three independent experiments.
b Fold increase compared with either the WT or As20.3rev.

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REFERENCES


6. Fairlamb, A. H., and A. Cerami. 1992. Metabolism and functions of trypano-


10. Haimer, A., C. Brochu, P. Genest, B. Papadopoulou, and M. Ouellette. 2000. Amplification of the ABC transporter gene PGPA and increased trypanothione levels in potassium antimony tartrate (SbIII) resistant Leish-


11. Haimer, A., C. Guimond, S. Pilote, R. Mukhopadhyay, B. P. Rosen, R. Poulin, and M. Ouellette. 1999. Elevated levels of polyamines and trypano-

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