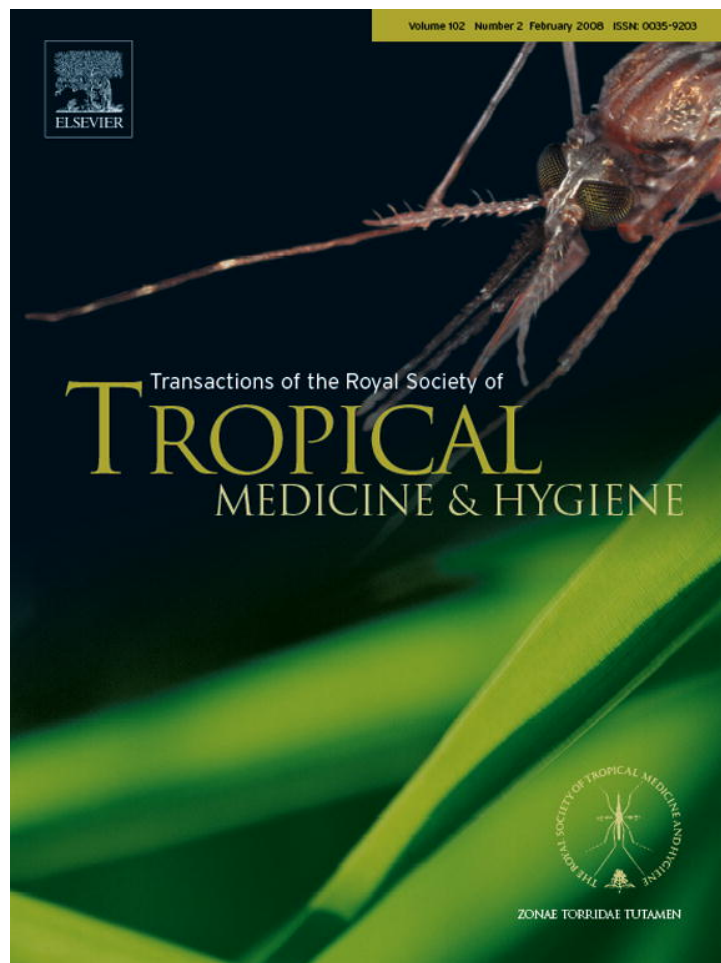


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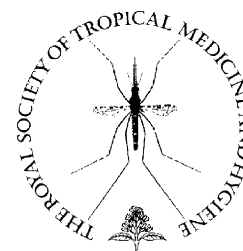


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MINI-REVIEW

Polymorphisms of *cpb* multicopy genes in the *Leishmania (Leishmania) donovani* complex

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 COOH-terminal extension;
 Polymorphism

Summary In leishmaniasis, cysteine protease b (*cpb*) multicopy genes have been extensively studied because of their implication in host–parasite interactions. In the *Leishmania donovani* complex, responsible for visceral leishmaniasis, a set of interesting polymorphisms has been revealed, such as copy sequence or expression according to the parasite's life stage. The single nucleotide polymorphisms observed among these copies could be related to clinical characteristics such as dermatropic versus viscerotropic status. CPB COOH-terminal extension (CTE) is mainly responsible for genetic variability among the copies and appears highly immunogenic. These results suggest that further study of the role of CPBs, especially CTE in clinical outcome, is warranted.

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The leishmaniasis, caused by protozoan parasites of the *Leishmania* genus (Kinetoplastida order, Trypanosomatidae family) are an important health problem in many regions of the world, especially the *Leishmania (Leishmania) donovani* complex, which is responsible for visceral leishmaniasis (VL), the most severe pathology. A great deal of research on *Leishmania* has focused on cathepsin L-like cysteine protease b (CPBs) because of their implication in host–parasite interactions (Mottram et al., 1997). These proteases belong to the CA clan (or papain-like, which are found in a number of organisms from prokaryotes to mammals) and the C1 family (Mottram et al., 1997). The C1 family contains two subfamilies, identified as cathepsin L-like (CPA, coded by a unique gene; and CPB, coded by multicopy genes) and cathepsin B-like (CPC, coded by a unique gene).

CPBs have key roles in infection, expression of disease and in effective autophagy (catabolic system, whereby eukaryotic cells can degrade and recycle proteins and organelles). In addition, CPBs present other interesting properties, such as: (1) their differential expression (CPB isoforms are mainly overexpressed in the amastigote stage); and (2) their gene organization (multicopy genes with a variable number of copies) (Mottram et al., 1997). As an example, *L. mexicana cpb1* and *cpb2* are predominantly expressed in metacyclic promastigotes, whereas *cpb3–cpb18* are expressed in amastigotes and *cpb19* is not transcribed. The functional properties and gene organization of *cpb* strongly suggest an archival library of variant genes such as *vsg* genes within *Trypanosoma brucei* or *var* genes within *Plasmodium falciparum*.

As already described, the evolutionary history of CPBs is essentially made up of gene duplication events, and differences in copy number and nucleotide sequences exist among the different *Leishmania* species. For example, *L. mexicana cpb* are located in a single locus of 19 copies arranged in a tandem repeat and polycistronically transcribed, whereas

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the *L. major* genome is composed of eight copies (Mottram et al., 1997). The *L. donovani* complex (responsible for VL) cluster contains five copies, which are arranged in a tandem repeat of 2.9 kb and the 5'UTR and intergenic regions are conserved in the cluster (Mundodi et al., 2002). *cpb* codes for three regions: a pre-pro-domain [from amino acid (aa) 1 to aa 124], a mature domain (from aa 125 to aa 342), and an unusually long COOH-terminal extension (CTE) rich in proline, serine and/or threonine residues (varying in size depending on the CPB isoform), which is specific to the trypanosomatids (Hide et al., 2007; Mottram et al., 1997) [CTEs of plants have no homology with CTEs of trypanosomatids and vertebrate CPBs have no CTEs (Mottram et al., 1997)]. The CTE is processed to give the mature cysteine protease. Concerning the *L. donovani* complex, CPBs have specific patterns such as potential epitopes and N-glycosylation sites (Hide et al., 2007). Among the three known CPBs (*cpbA*, *cpbE*, *cpbF*) of this complex, *cpbA* is genetically closer to other *Leishmania cpb* than to *cpbE/F* because of the CTEs. Indeed, *cpbE* (*L. infantum*) and *cpbF* (*L. donovani*) revealed a shorter CTE that has no homology with other *Leishmania* CTEs, and this CTE contains potential specific epitopes (HLA-A2 and HLA-DR1) (Hide et al., 2007). The cysteine protease activities of these three isoforms (CPBA, CPBE and CPBF) have been compared, and CPBA (which is predominantly expressed in promastigotes) and CPBF cleaved gelatine, whereas CPBE was found to be inactive in gelatine assays (Mundodi et al., 2002). *cpbE* and *cpbF* are very similar except for a sequence of 13 amino acids (GVLTSAGDALNH) in the 3' part of the mature domain (Hide et al., 2007; Mundodi et al., 2002). This sequence, absent for *L. infantum* but conserved among *Leishmania* CPBs, contains the His₁₆₃, which belongs to the catalytic triad (Cys₂₅-His₁₆₃-Asn₁₈₃). This catalytic triad is associated with the protease activity of all papain-like proteases. Comparing dermatropic versus viscerotropic *L. infantum*, single nucleotide polymorphisms in *cpbE* of dermatropic strains led to amino acids that are identical to those of *L. mexicana* CPBs, a dermatropic species (Hide et al., 2007). The real CTE function is not known, but it appears not to be essential for activation or activity of the enzyme (as proteinases that lack the full extension are active) or for its intracellular trafficking (Mottram et al., 1997; Rafati et al., 2003). Nakhaee et al. (2004) have shown

the importance of *L. infantum* CTE as a target of immune response in canine leishmaniasis, and there is some evidence to suggest that the CTE has a role in immune evasion (Rafati et al., 2003). CTE appears highly immunogenic, as assessed by the presence of antibodies and the presence of CTE-reactive peripheral blood mononuclear cells, and may play a role in diversion of host immune response. Regarding all these results, it is necessary to further study the role of CPBs, and especially the CTE in the viscerotropic character of the *L. donovani* complex.

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