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IDENTIFICATION AND PURIFICATION OF A 72 kDa ANTIGEN OF *LEISHMANIA BRAZILIENSIS BRAZILIENSIS* PRESENT ON THE SURFACE AND IN THE CYTOPLASM OF THE PROMASTIGOTES AND ITS SPECIFIC RECOGNITION BY SERA FROM MUCOCUTANEOUS LEISHMANIASIS PATIENTS.

Kutner, S. Pellerin, P., Brenière, S.F., Desjeux, P., Dedet, J.P.
Instituto Boliviano de Biologia de Altura, c/o Embajada de Francia, Casilla 824, La Paz, Bolivia.

Several studies on surface antigens of Old and New World *Leishmaniae* have assessed the presence of a common major glycoprotein of about 62-65 kDa, termed as gp63. This glycoprotein represents 80% of the total surface proteins, displays proteolytic activity and plays a key role in both virulence of promastigotes and their interaction with the host cells (1, 2, 3). However, a recent work in our laboratory showed that promastigotes of *Leishmania braziliensis braziliensis* (*L.b.b.*) harbour at their surface a specific major antigen of 72 kDa which does not cross-react with kala-azar and chagasic sera (4). We have already confirmed that this 72 kDa surface component is different by peptide digestion profile from the gp63 of *Leishmania donovani chagasi* (*L.d.c.*).

Specific recognition of the 72 kDa surface antigen by sera from mucocutaneous leishmaniasis patients

More than 30 sera obtained from bolivian patients suffering from cutaneous and mucocutaneous leishmaniasis were used to precipitate Nonidet P40 (NP40) proteins extracted from surface iodinated *L.b.b.* promastigotes. All sera recognized predominantly the 72 kDa antigen and

some of them precipitated high molecular weight proteins ranging from 150 to 200 kDa. The intensity of the band corresponding to the 72 kDa antigen was independent of the antisera title, of the clinical manifestation and of the geographic origin of the patient. On the opposite, sera from visceral leishmaniasis children did not precipitate the 72 kDa glycoprotein. This specificity was confirmed by a pulse-chase experiment, using an anti-L.b.b. hamster serum and NP40 extracts from non-labelled L.b.b. and L.d.c. promastigotes and **Trypanosoma** cruzi epimastigotes. A ten times excess of non-labelled L.b.b. extract inhibited 90% of 72 kda ^{125}I protein precipitation by the hamster antiserum, while the same quantities of L.d.c. and T. cruzi only gave 40 and 30% of inhibition, respectively. Thus, recognition of the major surface antigen from L.b.b. appears to be specific of anti-L.b.b. sera and could be used as a diagnostic test for mucocutaneous leishmaniasis.

Identification and purification of a 72 kDa cytoplasmic glycoprotein analogous to the major surface antigen

Proteins from surface iodinated L.b.b. promastigotes were extracted and separated by partitioning in the detergent Triton X114. Immunoblotting of the extracted proteins, using homologous antisera, showed recognition of two 72 kDa antigens : the first one, present in the detergent phase, was radioactively labelled, indicating so its presence on the promastigote surface, the second one, present in the aqueous phase, was non-labelled. The ampiphilic and hydrophilic antigens share common antigenic determinants inasmuch as monospecific

antibodies which recognized the ampiphilic labelled proteins reacted with the hydrophilic antigen. The identity of both proteins was confirmed by the homology obtained in their peptide digestion profiles and by the fact that both displayed proteolytic activity at neutral pH, as proved by zymogram assay. The hydrophilic glycoprotein seemed not to be generated during the Triton X114 extraction, it is present in large quantities in the promastigote cytosol and may play an important role in macrophage infection. The ampiphilic and hydrophilic 72 kDa antigens were further purified by a two-steps chromatography on Concanavalin A-agarose and DEAE-cellulose columns, they are actually under study to precise the nature of their homology.

Similarly, we demonstrated the presence of a cytoplasmic 63 kDa glycoprotein in L.d.c. promastigotes.

The coexistence of two cytoplasmic proteases analogous to the major surface antigens of L.b.b. and L.d.c. opens a perspective field of research. Their relation to immunogenicity and virulence remains to be elucidated and needs more investigation.