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SPECIFIC RECOGNITION BY SERA FROM MUCOCUTANEOUS LEISHMANIASIS PATIENTS OF 72 kDA ANTIGENS OF LEISHMANIA BRAZILIENSIS

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Leishmania braziliensis, surface antigen, 72 kDa.

Previous works in our laboratory demonstrated 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 (Legrand et al., 1987). We already confirmed that this 72 kDa surface component is different by peptide digestion profile from the gp63 of Leishmania donovani chagasi (L.d.c.).

35 sera from bolivian patients suffering from cutaneous and mucocutaneous leishmaniasis recognized predominantly the 72 kDa antigen. 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 dit 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 no-labelled L.b.b. and L.d.c. promastigotes and Trypanosoma cruzi epimastigotes.

Extraction and detergent separation of surface proteins from L.b.b. promastigotes showed recognition by homologous sera of two 72 kDa antigens :

- an ampiphilic glycoprotein present on the promastigote surface,
- an hydrophilic glycoprotein present in large quantity in the promastigote cytosol.

Both shared 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.

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MONOCLONAL ANTIBODIES TO PROTEIN PREPARATION FROM TRICHINELLA SPIRALIS AS A TOOL FOR ELISA DETERMINATION OF SPECIFIC ANTIGENS.

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Trichinella antigens, Monoclonal antibodies, ELISA-test.

Protein preparation consists of proteins of molecular weight range from 20 to 60 kD was isolated from T.spiralis larvae by gel-filtration on the G-200 Sephadex, and used as an antigen for immunization. The preparation was marked as a protein fraction II (PF-II) and contained two major proteins of molecular weight 43 and 37 kD. The spleens from BALB/c mice, immunized with PF-II were used to get spleenocytes for fusion with the cells of mouse myeloma X63.Ag 8.653 (subclone $P3O_1$). Five hybridoma clones were raised up, which produced monoclonal antibodies (MAB) against PF-II. All of them were of IgG1 isotype. Immunoblotting analysis showed, that all of the MAB recognize the protein of 43 kD. MAB were used for antigen determination in biological fluids by the method of competitive ELISA. The sensitivity of test was about 100-500 ng/ml. These MAB are considered as a perspective tool for immunosorbent preparing for isolation of pure fractions of Trichinella antigens, which would be useful for assemblage of immunodiagnostic kits for detection of trichinellosis both in humans and animals.