STUDIES ON SURFACE PROTEINS OF LEISHMANIA PROMASTIGOTES

By

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D E C L A RAT ION

I, Harmanjeet Jamnadass Nee Sharda, hereby declare that this is my own work and it has not been presented to any other University.

H.J. ~~~

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CANDIDATE

This thesis has been submitted for examination with my approval as the Univers~_~y~Supervisor .

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SUMMARY

Protozoan hemoflagellates of the genus Leishmania. causes disease in man and animals in ~any parts of the world. In the past taxonomic cla~sification has been based on geographical and clinical criteria. This has led to some confusion and hence there has been an increasing urgency for taxonomic re-classification.

In the present study, the promastigote form of the parasites were cultured in RPMI 1640 medium. RPMI 1640 was found to be better than *NNN* medium and Schneiders medium.

The Leishmania prom'8.stigotes were labelled by 125_1 by lactoperoxidase cat&Iysed iodination. The total protein in the promastigote lysates and the *suy*-face labelled proteins were analysed by polyacrylamide gel electrophoresis (SDS - PAGE). The protein profile of H48, isolated from human was identical to that of SIB which was i~olated from the host P. martini (Sandfly). However H48 and Sl8 exhibited a protein profile diffe-rent from Sll which is a possible species of lizard leish~aniasis. The molecular weight of some Sll.proma-stigote lysate proteins a~d stirface-labelled proteins differed from those of h48 and S18. Identical protein

patterns (of the promastigote lysate proteins and surface labelled proteins) were obtained when ~. donovani species from different geographical locations l;e. Kenya (051), Honduras (024), Bengal (025) and a species of L. braziliensis mexicana(026) were ana1ysed together.

Almost all the nroteins that were surface labelled were also found to be 3H-labelled and periodic acid schiff stained. This suggests that most of the surface proteins have a carbohydrate moeity i.e. they are glyco~ proteins.

The surface antigen profiles of H48, S18 and S11 were studied. The surface antigens were identified by immunoprecipitation wit~ rabbit sera against promastigotes. The surface antigen profiles of H48 and S18 exhibited identical patterns. Three major surface antigens of molecular weights 66,000, 53,000 and 48,000 were i~entified. Sll however displayed a distinctly different surface antigen profile where only 58,000 and 25,000 were detected as major surface antigens. The major surface antigens were proteins with acidic pI values.

The surface antigen profiles of 024, 025, 026 and 051 were also studied. All these isolates displa-

yed identical surface antigen profiles. Surface iodination and b{osynthetic labelling coupled to immunoprecipitation revealed the. presence of 59,000, 66,000 and 43,000 molecular weight surface antigens. Binding to concanvalin A suggests a glycoprotein nature of these antigens. The 59,000 and 43,000 molecular weight proteins were shown to be parasite synthesized proteins. The results suggest there are cross7reacting surface antigens on geographically different isolates. Immunofluorescence and immonodiffusion studies on these isolates also suggested the presence of cross-reacting surface antigens.

In conclusion, the differences obtained in the total lysate protein and:~~rface protein, vatterns may prove useful as a biochemi~al tool for classification of Leishmaniastrains. However, ,investigations on a large number of strains that have also been characterized by other parameters are needed to clarify this possibility. The presence of cross-reacting surface antigens suggests that it may be possible to vaccinate against infection by several different strains of Leishmania promastizotes, using surface antigens isolated from a single Leishmania strain. However, further investigations on the biochemical and immunological characteristics of isolated surface antigens are required to test this possibility.

Parts of the work described in this thesis has been published or 'is to be published under:-

1) Proteins and surface proteins of Leishmania promastigotes and their possible relevance to the characterisation of strains.
Ramasamy, R., Jamnadass, H. and Hutinga, M. J. International Journal of Parasitology, Vol. 11, No.5, pp. 387 - 390 - 1981.

2) Surface antigens on a Kenyan strain of Leishmania donovani.
Jamnadass, H., R. Ramasamy, J. Shah, M. J. Mutinga and S. Kar. East African Medical Journal Vol. 60 No.4 (1983) 238 - 240.

3) Cross-reacting surface antigens on Leishmania promastigotes. R. Ramasamy, S. K. Kar, H. Jamnadass. International Journal of Parasitology, Vol. 13 (1983).