

REA 09 - Immunological mapping of the OMP H6PGA4 _RICRI and evaluation of an ELISA for early diagnosis of spotted fever

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Introduction:

Brazilian spotted fever, maculose fever or Rocky Mountain spotted fever is an acute febrile infectious disease caused by *Rickettsia rickettsii*, transmitted by tick bite. Timely diagnosis is an essential first step in providing proper patient care and in controlling transmission. Maculose fever diagnosis in Brazil is often treated based on clinical diagnosis and/or indirect immunofluorescence. As this disease is rare and has high mortality rates in Brazil, there is a need of a development assay for rapid laboratory diagnosis for this condition. The OMP H6PGA4_ RICRI of *R. rickettsii* is a highly immunogenic transmembrane protein.

Objective:

Study the immunochemistry of OMP using a peptide library and serum of patients and use the most reactive to develop an ELISA-peptide for the diagnosis of the human disease.

Methodology:

A library of 84 peptides with 15 mer in length covering the extension of 429 amino acids of the H6PGA4 outer membrane protein and containing overlapping sequences of nine amino acids were synthesized by F-moc technique. The peptides were chemically bound to a cellulose membrane and reacted independently with patient's sera (n=5). A peptide enzyme-linked immunosorbent assay was used to confirm the reactivity and cross reactivity using a panel of 20 sera.

Results:

Eight distinct epitopes were mapped by this method. Four of the epitopes were located in the outer side of the transmembrane domains of mature processed protein. Three of these epitopes were present on both *R. prowazekii* and *Rickettsia typhi*. The analysis of ROC

curve indicated that the E4 and E5 peptides were the most immunogenic, with a specificity of 90% and sensitivity of 94% . .

Conclusion:

The IgG-ELISA-peptide test has high sensitivity, and, because is quick and easy to perform, be good confirmatory screening test for acute spotted fever infection.

Keywords: Maculouse fever; Peptide-ELISA; Diagnostic