

IDENTIFICATION OF MOLECULES ON THE SURFACE AND APICAL COMPLEX OF *Plasmodium berghei* OOKINETES THAT PARTICIPATE IN THE INVASION OF *Anopheles albimanus* AND *Anopheles pseudopunctipennis* MIDGUTS

Argotte Ramos, R. S., Lecona Valera*, A. N., Alvarado Delgado, A., Rodríguez López, M. H., Rodríguez Gutiérrez, M. C.**

Departamento de Patogénesis Molecular, Lab. 1 PB CISEI, Instituto Nacional de Salud Pública (INSP).
Av. Universidad 655, Sta. Ma. Ahuacatlán, 62508, Cuernavaca, Mor. México.
anlecona@hotmail.com*, mrodri@correo.insp.mx**

Malaria parasite transmission-blocking control strategies within the mosquito vector require an adequate understanding of the parasite/mosquito interaction at the molecular level. *Plasmodium* parasites development in the *Anopheles* mosquito vector is essential for transmission to humans. In the mosquito, midgut invasion by *Plasmodium* ookinetes is a critical step for establishment of the infection [1]. Micronemes are sub cellular organelles present in all invasive forms of *Plasmodium* including ookinetes, and their proteins are involved in cellular invasion. [2] To obtain and separate micronemes from *Plasmodium* ookinetes, a whole extract of culture ookinetes of *Plasmodium berghei* was fractionated in a sucrose discontinuous gradient (from 0.25 to 2.0 M). The fractions were analyzed by EM and polyacrilamide gel electrophoresis. By EM, Fraction F2 contained micronemes, and by unidimensional electrophoresis one band of ~200 KDa was visualized and cut-off to generate polyclonal antibodies using BALB/c mice. Polyclonal antibodies designated as R2 and R5 were probed by Western-blot using fraction F2 as antigen and protein extracts of whole ookinetes. EM immunolocalization using ookinetes of *P. berghei* was carried out with antibodies R2 and R5. WB showed three bands of approximately 42, 55 and 66 KDa under reducing conditions and only one band >200 KDa under non-reducing conditions. By EM immunolocalization, R2 and R5 antibodies reacted with proteins from ookinete micronemes. By two dimension gels (2D), spots of proteins were identified by Coomassie staining and Western blot with R2. Two spots recognized by WB with molecular weights of 42 KD (PI~5.1) and 55 KD (PI~5.4) were cut-off from the Coomassie gel (2D) and sent to obtain the amino acid sequence. Identification of these two proteins from *P. berghei* ookinetes could contribute to develop blocking transmission vaccines to block vector transmitted diseases such as malaria [3].

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RESPONSIBLE: *DRA. MARIA DEL CARMEN RODRIGUEZ GUTIERREZ.*

CO-AUTORES: *M EN C. ROCIO SOLEDAD ARGOTTE RAMOS, (INVESTIGADOR).*

M EN C. ALBA NERI LECONA VALERA (INVESTIGADOR).

M EN C. ALEJANDRO ALVARADO DELGADO (INVESTIGADOR).

DR. HUMBERTO LANZ MENDOZA (INVESTIGADOR).

DR MARIO HENRRY RODRIGUEZ GUTIERREZ (INVESTIGADOR).

anlecona@hotmail.com*, mrodri@correo.insp.mx**

INSTITUCION: *INSTITUTO NACIONAL DE SALUD PUBLICA.*

DIRECCION ACADEMICA: *AVENIDA UNIVERSIDAD 655 COLONIA STA. MARIA, CUERNAVACA MORELOS.*

TELEFONO: *(777)3 29 30 00 EXT 2362/2361*

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