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Synthesis and evaluation of monoclonal antibody against *Plasmodium falciparum* merozoite surface antigen 2

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Background: Several methods such as SDS-PAGE analysis are frequently used for monitoring the recombinant proteins during the expression and purification. As monoclonal antibodies are extremely specific, they can be used in association with Western blotting for confirming the expression of MSP-2 proteins of different domains and also to assess their quality and purity.

Methods: Mice were immunized with a schizont extract, to stimulate the immune system to make antibodies against different antigens of the late stage parasite, including the production of antibodies against different domains of *P. falciparum* MSP-2. B lymphocytes of immunized mice were extracted from the spleen and the fusion was performed using NS-1 myeloma cells and the hybridoma cells were assayed by ELISA either with a schizont extract or different domains of MSP-2 and/or by IFAT with whole schizont preparation. Fusion of NS-1 and spleen cells was performed. The positive hybrids were cloned and ELISA was applied against different dilutions.

Results: Overall from all 7 fusions performed 243 clones were grown in 96 wells plates as they were detected positive against crude schizont extract and only 10 clones were finally detected positive against different domains of the MSP-2 recombinant protein after sub-cloning in tissues culture flasks and before freezing. ELISA was performed to detect the positive hybrids against crude schizont extract by which the highest frequency to crude schizont extract was found for the supernatant of the hybrids produced in fusion number 3 (66 out of 315 hybrids). The supernatant of both B5 and F1 hybridoma cells were more positive against domain 2 of the MSP-2 recombinant protein in Western blotting test. Western blotting results showed that, different domains of the MSP-2 recombinant protein and also the MSP-2 of the *P. falciparum* parasite were recognized by some of the positive clones and also immune sera.

Conclusion: Bringing together all the results of this study it has been confirmed that some clones have recognized both schizont extract and different domains of the MSP-2 recombinant protein and therefore confirming the quality of the MSP-2 domains.

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Cutaneous leishmaniasis caused by *Leishmania infantum* in Turkey: reports of two cases diagnosed with genotyping and protein fingerprinting

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Background: Cutaneous leishmaniasis (CL) is endemic in mainly the southeastern regions of Turkey. The causative agent in most CL cases is predominantly *Leishmania tropica*, whereas *L. infantum* is mainly identified in patients with visceral leishmaniasis. However, there are few reports from Turkey that indicates *L. infantum* as the causative agent of CL. Here, we report two CL cases from Osmaniye and Hatay provinces located in eastern Mediterranean region in Turkey.

Methods: Normal 0 21 false false false TR X-NONE X-NONE MicrosoftInternetExplorer4

Samples obtained from the skin lesions of the patients were initially stained with Giemsa and cultivated in NNN medium. Examination of the smears and culture materials revealed *Leishmania* amastigotes and promastigotes, respectively. The promastigotes (MHOM/TR/2012/CBU15 and MHOM/TR/2012/MK05) obtained from the culture of both patients were used for further molecular analyses. Real-Time PCR analysis targeting the ITS-1 region in the SSU of rRNA were conducted to determine the *Leishmania* species of the cases.

Results: The results demonstrated that both samples were *L. infantum*, which was confirmed by protein fingerprinting analyses conducted by MALDI-TOF.

Conclusion: It is concluded that application of molecular methods determine the causative agents of infectious diseases more precisely than conventional methods, which is essential for correct diagnosis, effective treatment and prevention of severe epidemics (The present study is funded by TUBITAK-Project No: 111S179).

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