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A T-cell assay based on Antigen B multiepitope peptides for the diagnosis of cystic echinococcosis

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Background: The diagnosis and clinical management of human cystic echinococcosis (CE) is based on imaging examination and serology. However, currently, the serological tests used are still imperfect. Therefore, improved diagnostic systems and identification of new biomarkers are needed to defeat CE. We recently set up a whole blood (WB) assay by analyzing the Interleukin (IL)-4 response to the native Antigen B (AgB) of *Echinococcus granulosus*. However, the AgB is encoded by a multigene family with at least 5 major gene clusters coding for 5 putative isoforms. Moreover, the

procedure to obtain the native protein is difficult. Thus, the aims of this study were: i) to generate multiepitope synthetic peptides spanning the sequence of the 5 AgB isoforms and ii) to determine if the selected peptides may be used to increase the diagnostic accuracy of the WB assay for CE diagnosis based on IL-4 detection.

Material/methods: 39 CE patients and 21 healthy donors (HD) were enrolled. The peptides corresponding to each isoform were combined to obtain 5 pools. A pool containing all the peptides was prepared (total pool). The WB was overnight stimulated with the peptide pools and IL-4 levels were measured by an high-sensitive enzyme-linked immunosorbent assay.

Results: IL-4 levels were significantly higher in CE patients compared to HD when WB was stimulated with the pools corresponding to the isoforms AgB1 ($p=0.008$), AgB2 ($p=0.05$), AgB4 ($p=0.04$) and with the total pool ($p=0.02$). Based on these results, we performed a Receiver Operator Curve (ROC) analysis to evaluate IL-4 specific response potentials for CE diagnosis. Significant area under curve (AUC) results were obtained for all the peptide pools evaluated. The best results were found using the total pool. For scoring purposes we chose a cut-off point to maximize the test performance: a IL-4 level ≥ 0.32 pg/ml predicted CE with 51.3% sensitivity and 95% specificity. Moreover, the IL-4 levels were significantly increased in CE patients with active cysts compared to patients with inactive cysts, when WB was stimulated with the total pool ($p=0.002$). An additional ROC analysis showed significant AUC results (0.80; $p=0.003$), identifying a cut-off point of 0.6 pg/ml which predicted active cysts diagnosis with 73.3% sensitivity and 85% specificity. A significant and moderate positive correlation was found between the IL-4 levels induced by the total pool and the native AgB, used as control ($p= 0.0004$; $r_s= 0.54$).

Conclusions: For the first time we show that the AgB1, AgB2 and AgB4 are the immunogenic AgB isoforms in whole blood-based tests. Moreover, the IL-4 response induced by the total pool significantly associated with CE. These data, if confirmed in a larger cohort, offer the opportunity to develop new diagnostic tools for CE based on a standardized source of AgB as peptides.