PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RECOMBINANT CATHEPSIN L-A (rFgCatL-A) OF FASCIOLA GIGANTICA

LALITA INTHAKANOK

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Cathepsin L-A (CatL-A) is a major protein found in the ES antigens of Fasciola gigantica and is a target for diagnosing fasciolosis. There has been a study to produce recombinant CatL-A (rFgCatL-A) in E. coli in the form of fusion protein and purify it using metal-affinity chromatography. The purified rFgCatL-A was used to immunize experimental animals to produce monoclonal antibodies (monoclonal antibodies) and study the immune response to the immunization.

Three clones were obtained: 1D12, 2B11, and 13F5. Clone 2B11-E8-E3 was chosen for further study due to its high specificity. The monoclonal antibodies against rFgCatL-A were found to be specific to the ES and crude worm antigens of F. gigantica, indicating the potential of these antibodies in the diagnosis of fasciolosis.

In the study of the distribution of CatL-A in the tissue, it was found that monoclonal antibodies stained the Mehlis' gland and the heads of the spirochete in the testicle. It was also found that the monoclonal antibodies produced had a high specificity and were not cross-reactive with crude worm antigens of Eurytrema pancreaticum, Paramphistomum spp., and Schistosoma mansoni, indicating that these antibodies could be used in the diagnosis of fasciolosis.
PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANITBODIES AGAINST RECOMBINANT CATHEPSIN L-A (rFgCatL-A) OF FASCIOLA GIGANTICA

LALITA INTHAKANOK 4536197 SCEB/M
M.Sc.(ENVIRONMENTAL BIOLOGY)

THESIS ADVISORS : SUKSIRI VICHASRI-GRAMS, Dr.rer.nat., HANS RUDI GRAMS, Dr.rer.nat., ARAYA CHUSATTAYANOND, Ph.D.

ABSTRACT

Cathepsin L-A (CatL-A) is the major protein in the excretory-secretory (ES) products of Fasciola gigantica. Therefore, it was selected as the target for the development of a diagnostic tool for fasciolosis since this protein is present in all stages of the development of this parasite.

The recombinant protein (rFgCatL-A) was expressed in Escherichia coli as a fusion protein and purified by metal-affinity chromatography. It was then used for immunization of animals to develop monoclonal antibodies (MoAb) and to study the immune response. The stable hybridoma clones; 1D12, 2B11 and 13F5 were generated. The limiting clone, 2B11-E8-E3 clone was analyzed for its sensitivity and specificity. This MoAb detected 0.8 ng of rFgCatL-A and specifically reacted with 30 kDa rFgCatL-A and native 28-29 kDa CatL-A in ES products and crude worm extract of F. gigantica. Immunolocalization by MoAb found CatL-A specifically localized in the epithelial lining of the gut. The polyclonal immune sera localized cathepsin L in addition in the Mehlis' gland and in the head of spermatozoa. The MoAb did not cross-react with crude worm extracts of related trematodes including Eurytrema pancreaticum, Paramphistomum spp., and Schistosoma mansoni. The results of this study show that MoAb 2B11-E8-E3 may be applied for the immunodiagnosis of fasciolosis.

KEY WORDS : FASCIOLA GIGANTICA/ MONOCLONAL ANITBODIES/ CATHEPSIN L-A

158 pp.