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CHUMPORN SOOWANNAYAN: APOPTOSIS IN YELLOW HEAD VIRUS INFECTED SHRIMP. THESIS ADVISORS: TIMOTHY W. FLEGEL, Ph.D., BOONSIRM WITHYACHUMNARNKUL, MD., Ph.D., SUKATHIDA UBOL, Ph.D. 118 P. ISBN 974-664-823-3

A study of apoptosis was conducted in several organs of yellow head virus (YHV) injected shrimp. The cells and tissues studied included hemocytes, lymphoid organs (LO), gills, hepatopancreas (HP) and others. Four detection methods were used. These were haematoxylin & eosin staining, 4,6-diamidine-2-phenylidole dihydrochloride (DAPI) staining, TdT-mediated dUTP nick end labeling (TUNEL) assay staining and DNA fragmentation analysis. All these methods were used with hemocytes while only the first two methods were used with other tissues. The percentage of apoptotic hemocytes detected with all the staining methods increased with time after YHV injection from about 12% at 36 hours post injection to about 36% at 46 hours post injection. DNA extracted from hemocytes at 50 hours post YHV injection was found to be degraded to give a ladder-like pattern of about 200bp intervals when sorted by size using agarose gel electrophoresis. In the LO, many apoptotic cells were observed in the functional lobes while only a few apoptotic cells were found in lymphoid organ spheroids (LOS). The number of apoptotic cells in the LO and other organs increased with time post injection of YHV, in the same manner as with hemocytes. An immunohistochemical study of YHV infected shrimp sections using a specific monoclonal antibody against YHV revealed positive reactions in various organs and tissues. In the LO at early stages of infection, positive reactions were first observed in functional lobes and not in LOS. At later stages of infection positive reactions were seen at the periphery of Type B and Type C LOS and uniformly in Type A LOS. Many apoptotic cells in Type B and Type C LOS of both YHV and control shrimp were found to be negative for YHV. These results suggested that the shrimp used may have been co-infected with another virus. As an adjunct to this study, many methods of making hemolymph smears were tested. The best results were obtained with hemolymph smears allowed to attach to slides before fixation with either modified Davidson's fixative or with 10% formalin in PBS or in shrimp salt solution (SSS). Primary shrimp cell cultures were also studied. Primary cultures of hemocytes were successfully established on Fisher-plus® slides using Grace's insect medium supplemented with 5% FBS. The cultures could be kept at room temperature for up to 9 months, but they did not proliferate and they were not susceptible to YHV infection as determined by gross cytopathology. Primary LO cell culture experiments were also conducted using several culture media and supplements. Among these, 2x Leibovitz L-15 supplemented 15% FBS, 5g/L NaCl, 1g/L glucose, and 10% shrimp meat extract (SME) gave the most promising results, but the cultures obtained could not be subcultured and were not suitable for viral inoculation experiments.