ROLES OF PROTECTION IN PIGS GIVEN AN ATTENUATED HOG CHOLERA VIRUS VACCINE-LPC-CHINA STRAIN.

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Hog cholera (HC) has been considered as not a serious problem in many countries since the attenuated live vaccine was widely used. Piglets with no or low maternal antibody receiving one dose of HC vaccine usually obtained solid immunity against homologous virulent virus challenge. Once immunity established, the duration is generally considered as a life long, even the level of serum neutralization antibody decreased to very low, if any, or non-detectable amount. A long-term persistent infection of HC vaccine virus in the vaccinated pigs has been considered. Resistance of immune animals to infection perhaps depends on either specific protection mechanism ; humoral and cell mediated immunity (CMI) or non-specific defense factors such as interferon. However, the exact mechanisms that are response for the resistance of an immune pig against HCV are not known. In this paper we describe that humoral and cellular immunity induced in the HC live vaccine inoculated pigs and related to the resistance against virulent HCV challenge.

Twenty-eighty of 32 pigs aged 9 weeks free of HC antibody were intramuscularly injected with one dose of HC live vaccine of LPC-China strain virus respectively. Four non-inoculated pigs served as control. On day 3 to 7 daily and 14 post-vaccination, two vaccinated pigs on each day were intramuscularly challenged with virulent HCV of ALD strain, containing 10^4 MLD, in order to test the establishment of resistance, and the other two vaccinated pigs were sacrified, whose blood leukocytes, spleens and retropharyngeal and mesenteric lymph nodes were collected as the sources of lymphocytes for CMI. Meanwhile on the above indicated days after vaccination and day 3 and 7 after challenge, blood leukocytes and lymphocytes of four pigs were also collected for leukocyte migration inhibition (LMI) and lymphocyte blastogenesis (LB) tests respectively. Four non-vaccinated pigs were served for the sources of control lymphocytes and control of the challenge.

For measurement of LB, quadriplicate cultures, each containing 2 x 10^6 in 96 well microplates were supplemented with two concentrations of inactivated HCV antigen (10^3 and 10^4 $TCID_{50}$) and without antigen. Cultures were incubated at 37°C for 96 hours in a humified 5% CO_2 atmosphere, then 0.5 uCi ^3H thymidine was added to each well and the cultures further incubated for 18 hours. Cells were harvested, washed and counted. A lymphocyte stimulation index (SI) evaluted statistically was used as a measure of LB and determined by the ratio average cpm with antigen : average cpm without antigen. LMI technique was done in plastic petridishes (60 x 15 mm) with agarose medium consisting of Eagle's Minimal Essential Medium, 1 % agarose and 20 % fetal calf serum. Six wells (4-mm diameter) were made in each plate. Washed leukocytes suspension (10^8 cells/ml) from vaccinated or non-vaccinated pigs was divided into 2 tubes, 1 ml in each. An equal volume of inactivated HCV antigen or medium without antigen was added to the tubes respectively. The mixtures were agited and incubated at 37°C for one hour. After incubation, 25 ul of the mixture was filled to each well ; 4 replicates of each specimen were used. The plates were incubated at 37°C in an atmosphere of 5% CO_2 with water vapor for 48 hours. Results were recorded as percentage of inhibition and calculated as follows :

$$\% \text{ LMI} = [1 - (A/B) / (C/D)] \times 100$$

where A : migration area of leukocytes ffom vaccinanated pig incubated with antigen ; B = migration area of leukocytes from vaccinated pig incubated without antigen ; C = migration area of leukocytes from control pig incubated with antigen ; D = migration area of leukocytes from control pigs incubated without antigen. Inhibition of 20 % or greater in average of 4 replicates of each specimen was considered as positive CMI response.

Clinically one of 2 pigs challenged on 3 days post-vaccination (DPV) died of typical HC as did control pig. The other and all pigs challenged on 4, 5 and 6 DPV survived with a transient fever response (40-41°C) for 2 to 3 days. However, pigs challenged on 7 and 14 DPV survived without any clinical reaction.

CMI determined by LB was not significantly detected in all vaccinated pigs within 14 DPV no matter peripheral or tissue lymphocytes were tested. The SI of lymphocytes obtained from most pigs were ranging from 0.8 to 1.5 except 2 pigs with SI of 2.5 and 3.4 on 6 and 7 DPV respectively. However, significant CMI response determined by LMI technique were observed in two vaccinated pigs with the highest SI ; one on 6 DPV and the other on 7 DPV ; the % LMI was 20.3 (SI : 2.5) and 27.0 (SI : 3.4) respectively. Two techniques used for determining the response of CMI were well correlated, although the SI was not significant. CMI was not also observed in those pigs within 7 days after challenge determined by LMI technique.

The appearance of seroconversion varied from pigs to pigs : detectable amount of serum antibody (1 : 2 to 1 : 3 in titer) appeared in some pigs on 3 DPV as compared to the pre-vaccinated serum samples. Serum antibody titer did not increase significantly within 14 DPV. After challenge, those pigs challenged on 3 to 7 DPV did not show secondary response, but pigs challenged on 14 DPV showed approximately a 3 fold increase in titer (1 : 4 to 1 : 16) on day 7 after challenge.

The negative results of CMI in most of vaccinated pigs indicated that CMI may play little, if any, important role in the protection against virulent virus challenge than humoral immunity as evidenced by the results of challenge in the first group (3 DPV) ; pig with no detectable serum antibody died, but the other with low (1 : 3) but detectable survived. However, the other factors might be also involved in the protection such as interferon should not be excluded for the resistance of the vaccinated pigs acquiring in the early stage.

Conclusions :

Pigs immunized with HC attenuated live vaccine acquired resistance against virulent virus challenge as early as 3 DPV. In general, CMI was not detectable in most of pigs within 14 DPV and 7 days after challenge using LB and LMI techniques. Experimental data suggest that humoral immunity may play more important role than CMI in the active immunization condition.

Selected references : Shimizu, M. and Pan, I.C., : AJVR 1977, 38 : 27 ; Corthier, G. : AJVR 1978, 39 : 1841 ; Torlone, V., Titoli, F., and Gialletti, L. : Life Science 1965, 4 : 1707 ; Lin, T.C., Shieh, C.M., Chen, Y.C., Chen, C.C., Lee, C.S., Lai, S.S. : Prov. Res. Inst. Anim. Hlth. Exp. Rep. (Taiwan) 1969, 6 : 11