HUMNORAL AND C.M.I. STUDIES ON PIGS AND PIGLETS INFECTED WITH DIFFERENT FIELD ASF ISOLATES.

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INTRODUCTION

One of the primary objetives of African Swine Fever (ASF) research, is to produce an efective vaccine. The immunology of this infection is not well known. Eventhough, have been detected neutralizing antibodies in pigs infected with certain isolates of ASF. Some work has been done to study some immunological aspects of ASF infection, both in the humoral (De Boer, 1967) and the cellular response (Schimizu et al., 1977, Wardley, 1979, Sánchez-Vizcaino et al., 1981).

Some isolates will be useful for studing the immunology of ASF and hog cholera (HC) virus infection were undertaken to test the different response on pigs and piglets treated with high and low virulence ASF isolates.

MATERIALS AND METHODS

Adult pigs: A total of 25 adult pigs weighing between 20 to 40 kg were infected with ASF isolates and HC virus.

Baby pigs: In the same way, 25 baby pigs were infected with the same virus.

Control pigs: Five adult pigs were used as control animals.

Bleeding: All the pigs were bled every four days.

Inoculation virus: The inoculum used was made with their different isolates diluted in PBS with a final volume of 3 ml each. The inoculum titer was $10^{5,3}$ HAD/ml for Dominican Republican isolates, $10^{4,1}$ HAD/ml for Spain 79, $10^{6,1}$ HAD/ml for Brazil and $10^{5,8}$ HAD/ml for Lisbon 60.

ASF antigen for blastogenic assay: Viral antigen was prepared by growing ASF virus Spain 75 partially atenuated in an MS cell line (Sánchez-Vizcaino et al., 1981). The cytophatic effect was about 90-100%, the culture was harvested, centrifugated (400 g, 15 min.) and the supernatant was treated with UV light. Then hemadsorption test (Malmquist and Hay, 1960) was performed to prove inactivation.

Viremia and antibody levels: The viremia was evaluated by the hemadsorption test and the antibody levels were studied by indirect immunofluorescence (Sánchez Botija et al., 1970) and ELISA test (Sánchez-Vizcaino et al., 1979, 1981).

Rosset forming cells (RFC): Briefly, lymphocytes were incubated with 0,5 ml of 5% suspension of neuraminidase treated SRBC for 5 min at 37ºC, centrifuged (200 g, 7 min), left overnigth at 4ºC, resuspended and REC enumerated.

Blastogenic assay: Populations of T and B cells were obtained using Ficoll hypaque gradient. 3 ml of heparinized pig blood diluted (1:1) in PBS, pH 7,2, were layed over 3 ml. of Ficoll hypaque in a tube. After centrifuging (400 g, 30 min) at 4ºC cells populations at the interface were collected and cells were washed 3 times in PBS. The pellet was resuspended in RPMI-1640 with fetal calf serum to a final concentration of 25×10^4 cells per well. The cells and mitogen were incubated in a microplate for 72 hours or 96 for ASF antigen in an atmosphere of high humidity and 5% CO_2. In the lats 18 hours, the cells were pulse labelled with 1 uci/well for 3H thymidine. Cultures were then harvested using a Mash II and radiactive incorporation was measured by a scintillation counter. Results were expressed by cpm count of the wells receiving mitogen or antigen divided by the cpm count in wells incubated with cells and medium.

Mitogen: The mitogens used to induced blast transformation were phytohaemagglutinin (0,1 ug/ml) (PHA), pockeweed mitogen (0,1 ug/ml) (PWM) and LPS at final concentration of 0,5 ug/ml.

RESULTS

Adult and baby pigs were infected with one of the following: high virulence isolates (Lisbon 60 and Spain 79), low virulence isolates (Dominican Republic and Brazil) or HC virus.

Adult pigs: All the animals infected with either Dr or Br isolates survived after 15 DPI (maximum observed), in contrast the pigs infected with Lisbon 60 or HC virus dead. Until 4 DPI the response of the pigs were similar. After 4 DPI, the response of pigs infected with high virulence decrease and the pigs died. After 4 DPI, the pigs infected with lou virulence has increasing mitogenic response.

To determine if the decrease in blastogenic response of ASF infected pigs was a non-specific effect of viral infection, adult pigs were inoculated with HC virus. HC infected pigs had a moderate decrease to PWM and PHA mitogenic response and a marked decrease to LPS. In contrast, ASF infected pigs had a contrary response.

Bay pigs: All the animals inoculated with ASF isolates died between 4 and 9 DPI because they had a total depression of blastogenic response to all mitogens and ASF antigen. In contrast, all piglets infectd with HC virus survived.

Is interesting to note that age had an apparent marked effect on the clinical course of HC and ASF infections. Also, the piglets had a more uniform response than adult pigs to all the parameter studied.

DISCUSSION

The results of this work show that the differences in the clinical course, some aspects of the immune response and the pathological finding between low and hing virulence ASF isolates depended, not only on the isolates themselves but also on the age of the animals. The divergence of the clinical course of adult pigs inoculated with either high or low virulence ASF virus at 4 DPI was reported like an appearence of IgM at 4 DPI and IgG at 6 DPI (Sánchez-Vizcaino, in progress) and means that the onset of an immune response in pigs infected with low virulence ASF virus occurred at about the same time as the immune response in pigs infected with conventional viral agents.

The differences observed between the mitogenic response to the HC virus and ASF isolates could be due fact that these virus appear to affect different subpopulations of lymphocytes, suggesting that there is infection but not replication of ASF virus in the T lymphocytes.

The difference in mitogenic response of pigs infected with HC or ASF, could be that, ASF lesions occur in the cellular stromal areas of the lymph-nodes, while those of HC occur in the lymphonodules.

The results of this work suggest that an immune response is responsble for clinical recovery from ASF infection. Future work must identify the specific cell population and immunogen in volved in recovery.

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