Immunopathogenesis of Chicken Infectious Anemia Virus Isolated in Taiwan

Introduction

Chicken anemia virus (CAV) causes considerable worldwide economic losses in the poultry industry. CAV causes transient severe aplastic anemia due to destruction of erythroblastoid cells and generalized lymphoid atrophy with a concomitant immunosuppression in 2-4-wk-old chickens (Joiner et al, 2005). Increased susceptibility to various viral, bacterial, and fungal pathogens has been associated with impairment of the host immune response following CAV infection. CAV is a 23-25 nm virus containing a circular minus strand DNA genome. The pathogenesis of CAV infection has been elucidated by sequential virologic, pathologic, immunocytochemical, and immunologic studies of experimentally infected chickens. There is lack of information directly comparing the dose of CAV inoculation despite the applied importance of this knowledge. Effects were assessed by sequential clinical, pathologic, and morphometric histopathologic evaluations Therefore, the present work was undertaken with the aim to investigate the relationship among clinical symptom, mortality, lymphocyte depletion and antibody response.

Materials and Methods

Virus. The fourth passage CAV isolate 104-05 was used for experimental infection. CAV 104-05 was isolated from Taiwan replacement layer in 2015 with weak, depression and 0.25-1.5% mortality related to pale and anemia. A total of 5 condemned chickens with no external lesions but had gross thymic atrophy. The virus was isolated from thymus samples in MDCC-MSB1 cells in accordance with generally accepted procedures. CAV 104-05 titration was conducted in MDCC-MSB1 cells. Briefly, MSB1 cells in 24-well plates (2.5×10^5 cells/ml per well) were inoculated with 20 ul of 10-fold serial dilutions of CAV stock, four wells per dilution, achieving a titer of 10^6 tissue culture infective doses ($TCID_{50}$) per milliliter.

Experimental inoculation and sampling. Specific-pathogen-free (SPF) chickens were separated into three groups of 27 chickens. Groups were maintained under isolated conditions with food and water ad libitum throughout the experimental period. Chickens in groups 1 were inoculated at 1day of age by the IM with 10^4

$TCID_{50}$ of CAV 104-05. Group 2 were inoculated at 1day of age by IM with 10^5 $TCID_{50}$ of CAV 104-05. Group 3 served as uninfected controls. The body weight and blood samples for hematocrits were obtained from each bird at 7, 14, 21, and 28 days after inoculation. Statistical differences between the groups were evaluated by Student t-test. On days 7, 14, 21 and 28 after inoculation, serum samples were collected from five birds from each group, which were subsequently euthanatized and examined at necropsy. Samples of thymus, were stored at -85 ^{o}C for subsequent isolation of DNA for real-time quantitative polymerase chain reaction (qPCR). Samples of thymus were collected and fixed by immersion in 10% neutral buffered formalin for histopathologic evaluation. All samples collected were identified by individual chicken to allow assessment of correlations among parameters in individuals.

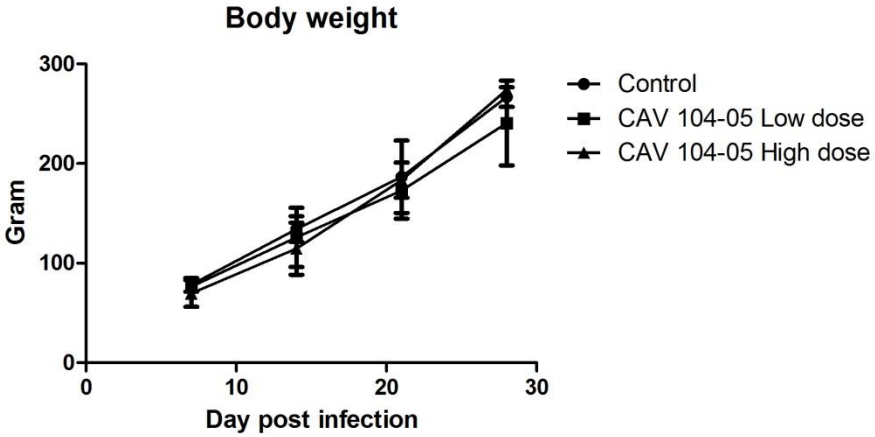
Serology. Sera were tested by competitive enzyme linked immunosorbent assay (ELISA) for specific antibodies against CAV according to the manufacturer's recommendations (Synbiotic Corporation, Inc.) using 100-fold dilutions of each serum sample. Optical densities obtained were evaluated statistically for possible differences within and between the groups by nonparametric analysis of variance. Since competitive ELISA was used, lower values indicate presence of antibody in sera.

Histopathology. Formalin-fixed sections of thymus were routinely processed, embedded in paraffin, and sectioned at 4 to 6 u. All sections were stained with hematoxylin and eosin (Gill's 2) using eosin phloxine counterstain and examined histologically. In addition to histopathologic examination, a digital photograph of a section of a representative thymus lobule from each bird was taken at 4X magnification. Morphometry was conducted using ImageJ software version 1.51 (public domain,http://rsb.info.nih.gov/ij/). The perimeter of the lobe was marked with the polygon selection tool. The color image was converted to an 8-bit black and white image and the threshold tool applied, adjusted to discriminate between darker lymphocyte-rich cortical regions in comparison to the lighter medullary regions. Two measurements were made from each lobe. First the total area of the lobe was measured. Second, the lymphocyte rich area (above threshold) within the demarcated lobule was measured. The cortex lymphocyte to parenchyma (CL/P) ratio was calculated by dividing the lymphocyte-rich area by the total area. This ratio was subjected to one-way analysis of variance (GraphPad Prism 6.00,

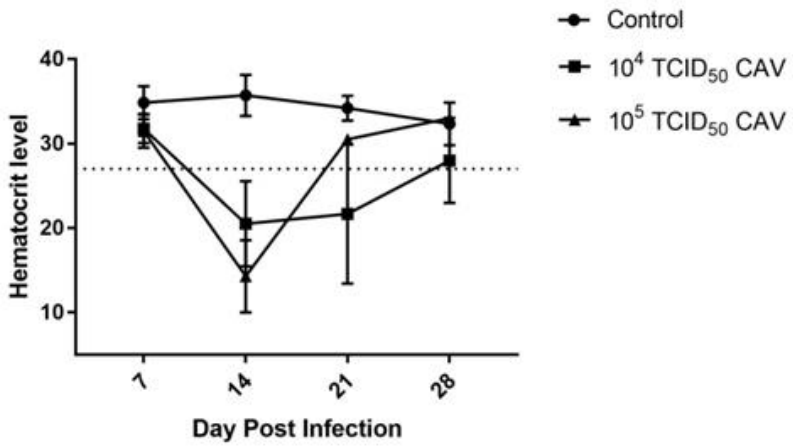
GraphPad Software, Inc.). Tukey multiple comparison test was used to compare groups with significant differences ($P < 0.05$).

Results

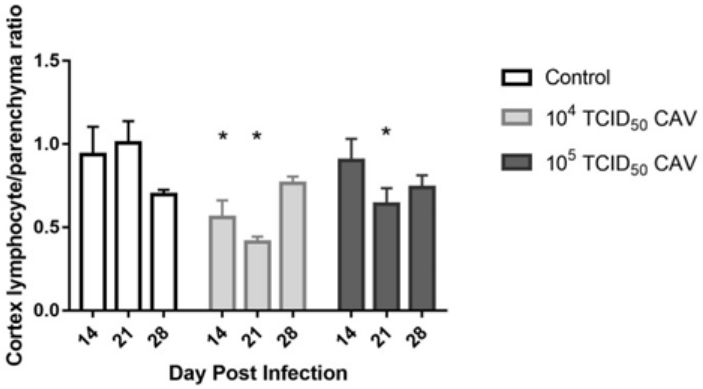
Clinical signs. As shown in Fig. 1, the inoculation of chickens with CAV 104-05 caused reduced weight gain, resulting in lower weights, in both the low and the high dose infected chickens as compared with the controls. Linear regression analysis indicated that the rates of weight gain of all three groups were significantly different from each other at a 95% confidence interval. The weights of low dose-inoculated chickens were significantly different ($P < 0.05$) from uninfected controls at 21 and 28 day post infection. The lowest weight gain rates were maintained throughout the experimental period in the low dose-inoculated group ($P < 0.05$).



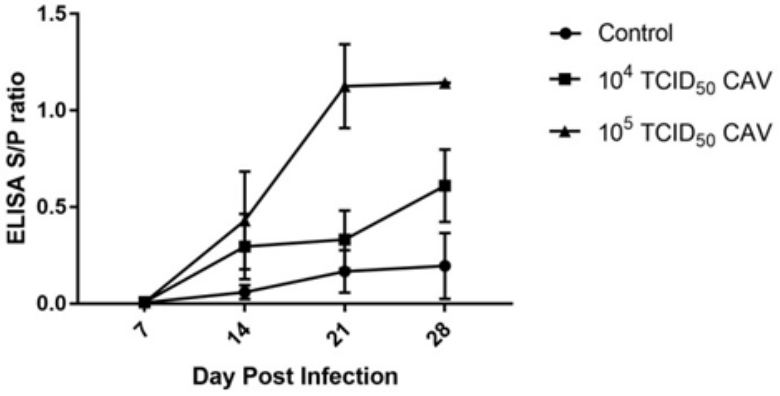
Compared with uninfected control chickens, low dose infected chickens showed significantly reduced hematocrits ($P < 0.05$) from day 7 PI throughout the experimental period (Fig. 2). These birds showed moderate to severe depression. The increased hematocrits observed at days 21 and 28 in the chickens of this group are most likely a consequence of dehydration due to reluctance to move to the water supply. High dose infected birds did not have significantly reduced hematocrits ($P < 0.05$) until day 14 PI, maintained low percentages through days 21, and recovered to normal hematocrits at day 28 PI. Disease was not apparent in this group.



Gross lesions and histopathology. Thymic atrophy, sometimes resulting in an almost complete visual absence of thymic lobes, was the most consistent lesion in the chickens inoculated with CAV 104-05 with the low dose. At day 14 PI, all birds of the low dose group showed severe thymus atrophy. At day 21 PI, hemorrhages in breast muscles were observed in 50% of the chickens of this group. High dose inoculated chickens did not have detectable gross lesions until day 21 PI. At day 21 PI, a less severe thymic atrophy was observed in one of two birds (approximately 50%) of that group. No macroscopic changes were detected in the bursa of any birds. The lymphocytic depletion in the thymus, expressed as the ratio of cortex lymphocytes to parenchyma (CL/P), observed at different time points after infection is shown in Figs. 3. This lymphocyte depletion was significant ($P < 0.05$) and most severe at day 14 PI in the low dose-inoculated chickens, while high dose infected birds did not show statistically significant differences to control chickens at this time. At day 28 PI, repopulation of the thymus with lymphocytes had occurred in both infected chicken groups, and no significant differences were detected between them and the controls.



Serology. CAV-specific antibody levels increased in chickens inoculated both the low dose and the high dose with isolate 104-05 (Fig. 4). No significant differences ($P < 0.05$) could be detected between the groups through day 7 PI. Antibody levels increased after day 14 in the high dose inoculated group, showing significantly higher values ($P < 0.05$) at day 14 PI. Low dose inoculated chickens increased their antibody levels after day 21 PI, showing significant differences ($P < 0.05$) at day 28 PI. The achieved levels did not differ significantly from the high dose group at that time point. The controls maintained a negative status throughout the experimental period.



Discussion

CAV isolate 104-05, obtained from commercial replacement layer showing clinical disease characterized by depressed, was pathogenic for SPF chickens when applied either with the high dose or the low dose. The chronologic changes observed in IM-infected chickens, clinical findings (signs, hematocrits, and weight gain rates), macroscopic lesions, and histopathologic changes in the thymus, are in agreement with findings by other authors. Our findings in different dose infected chickens are also in accordance with the partial information that is available. Similarly, 104-05 infected chickens of the present study did not show a significant reduction of weight gain through day 14 PI. In the present study, a reduction of the parameters occurred at a later time point and hematocrits achieved normal values at day 28 PI. To our knowledge, only partial information (Rosenberge et al, 1989) is available from other authors on these clinical changes between days 14 and 28 after low dose inoculation of 1-day-old chicken. Lymphocytic depletion in the thymus of low dose-inoculated chickens was still extensive at day 14 PI, and lymphocytic depletion in thymuses of high dose inoculated chickens was not assessed between

14 and 21 DPI. Interestingly, lymphocyte repopulation of the thymus occurred by day 21 PI in spite of the presence of the virus in this organ as demonstrated both by real-time PCR (Data not show). Perhaps the virus persists in thymic lymphocytes where it cannot be affected by immunoglobulins. When the cell finally dies because of apoptosis, the antibody acts, preventing most of the released virus from infecting new cells, allowing an efficient thymic repopulation. The virus concentrations decline only gradually, probably because a few cells get infected in spite of presence of antibody. Compared with the low dose of infection, chickens infected high dose with the same virus and the same dose did not show apparent signs of illness. High dose inoculation resulted in a less severe reduction of weight gain and hematocrit values, which also occurred at a later time point. These differences were in agreement with the gross and histopathologic changes, which were also less severe and delayed in the low dose inoculated chickens.

References

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