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ANTIBODY RESPONSES IN CHICKENS EXPERIMENTALLY INFECTED WITH LOW AND HIGH PASSAGED CHICKEN ANEMIA VIRUS ISOLATES

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SUMMARY

It has been shown that chicken anemia virus (CAV) which had undergone 60 and 123 passages in cell cultures (SMSC-1/P60, SMSC-1/P123, 3-1/P60 and 3-1/P123) were less pathogenic compared to low passaged CAV (SMSC-1 and 3-1) isolates. In this study, the ability of the isolates to induce antibody responses was studied using enzyme-linked immunosorbent assay (ELISA). All the isolates regardless of the number of cell culture passages that they had undergone elicited CAV antibody responses both at 16 and 30 days post inoculation. A CAV isolate, BL-5 that was not passaged in cell culture elicited higher antibody response than the cell culture passaged isolates. However, the differences in the average ELISA titres and the percentage of positive sera between the isolates were not statistically significant ($P>0.05$). The study showed that CAV isolates which had undergone repeated passages in cell culture are still immunogenic.

Keywords: chicken anemia virus, antibody response, enzyme linked immunosorbent assay

INTRODUCTION

Chicken anemia virus (CAV) is an economically important avian pathogen with a worldwide distribution (Bulow *et al.*, 1986; McNulty, 1991). The disease caused by the virus is characterized by aplastic anemia and generalized lymphoid atrophy with a concomitant immuno-suppression. Consequently this disease is frequently characterized by secondary bacterial infections, viral infections or vaccination failures (Engstrom and Luthman, 1984). It has been suggested that CAV exert its cytopathogenic effect by inducing apoptosis leading to depletion of cortical thymocytes in young chickens (Jeurissen *et al.*, 1989).

An immunological comparison of field isolates in the United States and Europe suggests that only one serotype exists in CAV (McNulty, 1991). Serum antibodies to CAV can be detected by using immunological tests such as virus neutralization (VN), immunofluorescence (IF) and immunoperoxidase staining and enzyme-linked immunosorbent assays (ELISA) (Chandratilleke *et al.*, 1991; Goodwin *et al.*, 1992; Lamichhane *et al.*, 1992; Todd *et al.*, 1999). The VN test is reliable and has been reported to be more sensitive than the IF test and ELISA. However, the test is expensive and time consuming. On the other hand, ELISA has several distinct advantages over the other detection methods as it requires no microscopic examination, is generally more sensitive than IF test and is designed for an automated system.

Serological detection of CAV is important since in some countries the disease had been controlled by vaccination of breeder chickens which are tested serologically before they come into lay, to ensure that they are CAV antibody-positive. In addition, maternal antibodies provide complete protection of young chicks against CAV-induced infectious anemia (Yuasa *et al.*, 1983). It has also been shown that chicks inoculated with CAV at one day of age developed protective neutralizing antibodies two weeks later (Otaki *et al.*, 1992). Only very low levels of antibody were needed to give effective protection from infection and that once CAV antibodies have been produced they persist for a long time.

A serological study on CAV carried out in different broiler and layer farms indicated a high prevalence of the virus in Malaysia (Rozanah *et al.*, 1995). Recently, we have isolated several CAV isolates from broiler chickens (Chowdhury *et al.*, 2002; Kono *et al.*, 2000). Some of the isolates have been characterized based on sequence analysis and pathogenicity studies (Chowdhury *et al,*. 2003; Hasmah *et al.*, 2004). However, the ability of the isolates to induce antibody responses in specific-pathogen-free (SPF) chickens is not known. The present study describes the antibody responses in chickens after inoculation with low and high passaged CAV isolates.

MATERIALS AND METHODS

Viruses

A total of seven different isolates were used in the study: these included two low passaged (SMSC-1 and 3-1) and four high passaged (SMSC-1/P60, 3-1/P60, SMSC-1/P123 and 3-1/P123) isolates (Chowdhury *et al.*, 2003) and a non-passaged isolate, BL-5 (Chowdhury *et al.*, 2002). The BL-5 isolate was prepared from liver homogenate sample of a field case of broiler chickens infected with CAV.

Chickens

Single comb White Leghorn embryonated SPF eggs were imported from Sunrise Farm Inc, Catskill, NY, USA. The SPF eggs were hatched and reared in an experimental isolation house throughout the experiments. Feed and water were provided *ad libitum*. One-day old SPF chickens were divided into three experiments with each experiment consisting of virus infected group(s) and uninfected group as control. In the first and second experiments, the infected groups were inoculated intramuscularly with 0.1 ml virus each containing 10^6 to $10^{7.5}$ $TCID_{50}$ of SMSC-1, 3-1, SMSC-1/P60, 3-1/P60, SMSC-1/P123 and 3-1/P123. For the third experiment, the infected group was inoculated intramuscularly with 0.2 ml of liver homogenate from BL-5 infected chickens. Serum samples were collected at 16 and 30 days post-inoculation (p.i.). The sera were separated from the blood samples and kept at -20°C until further use.

Enzyme-linked immunosorbent assay

The serum samples collected from infected and control groups of chickens were analyzed using CAV ELISA test kit (Kirkegaard and Perry Laboratories, Maryland, USA) following the method recommended by the manufacturer. The plate was read using an ELISA plate reader (Dynatech MR7000, USA) set at 410 nm. The CAV ELISA titer was calculated by the following equation, Log_{10} titre = 1.009 X Log_{10} S/P + 3.628. Titer is expressed based on the antilog of Log_{10} titre.

Statistical analysis

The differences of percentage positive samples between groups at 16 and 30 days p.i. were analyzed for significant variation at 5% level by χ^2 test.

RESULTS AND DISCUSSION

The average positive control absorbance was 0.517 whilst the average normal control absorbance was 0.135. Hence, the corrected positive control was 0.382. Based on the calculation formula, and the positive and negative control absorbance values, the serum sample absorbance of less than 0.269 resulted in a titer value of 1479. Samples with antibody titer less than 1479 were considered as negative for CAV antibody.

The percentage of positive serum samples was higher in chickens vaccinated with SMSC-1/P123 followed by those vaccinated with SMSC-1/P60 and then SMSC-1, both at 16 and 30 days p.i. (Table 1). On the other hand, the percentages of positive sera were higher in 3-1 group of chickens followed by 3-1/P123 and then 3-1/P60 groups, both at 16 and 30 days p.i. (Table 2). The results also exhibited that, at 16 days p.i., 50% to 63.6% of the collected sera were detected positive, whereas these percentages of positive samples were increased to 69.2% to 83.3% at 30 days p.i. However, the differences in the average ELISA titers and the percentage of positive sera between the groups were statistically not significant ($p>0.05$). In an earlier study, Todd *et al.*, (1998) found the presence of broadly similar CAV antibody by indirect IF antibody test in sera taken from 6-week-old chickens that had been inoculated at one-day-old with different isolates of CAV at different passage levels such as pathogenic isolates and isolates that had undergone 49 and 170 passages in cell culture. However, the levels of antibody were greater than levels found in chickens inoculated with a CAV that had undergone 320 passages in cell culture. In another study, Yuasa *et al.* (1983) detected neutralizing antibody in chickens at 21 days after inoculation with CAV at 1-day of age but could not detect the neutralizing antibody at 14 days p.i. The differences in the ability to detect antibody after inoculated with CAV isolates may be due to the difference of virus dose and/or techniques used to measure the antibody levels.

Between the two low passaged pathogenic isolates (SMSC-1 and 3-1), a higher percentage of positive samples were obtained with sera of chickens inoculated with 3-1 isolate at 16 and 30 days p.i. Among the high passaged isolates, the percentage of positive samples was found high in the case of SMSC-1/P123 isolate both at 16 and 30 days p.i. The percentage of positive samples was found to be the lowest in groups inoculated with 3-1/P60 isolate. The reason for this variation could not be ascertained. However, the low antibody level may not be a problem, because studies have shown that a very low level of antibody is required to give effective protection from infection (Otaki *et al.*, 1992).

After inoculation of the chickens with liver homogenate of BL-5 isolate, 70% of the collected serum samples were found positive at 16 days p.i. (Table 3). This percentage of positive samples was the highest compared to all other inoculated groups of chickens described above at 16 days p.i. No serum samples were analyzed from the BL-5 infected chickens at 30 days p.i. The ELISA antibody titers were also high in BL-5 isolate than that of other isolates. This might be due to the fact that BL-5 isolate was inoculated as processed tissue homogenate from the field sample without any experience

Table 1: Serum antibody titers of chickens following inoculation with SMSC-1, SMSC-1/P60 and SMSC-1/P123 isolates at 1-day of age

CAV isolate	16 days p.i.			30 days p.i.		
	No. of sera	No. of positive sera (%)[a]	Average titer ± SD (range)[a]	No. of sera	No. of positive sera (%)[a]	Average titer ± SD (range)[a]
Control	10	0 (0%)	30[b] (-87 to 209)	10	0 (0%)	61[b] (-108 to 251)
SMSC-1	9	5 (55.5%)	1569 ± 576 (933 to 2388)	12	9 (75%)	2085 ± 610 (1375 to 2946)
SMSC-1/P60	12	7 (58.3%)	1632 ± 430 (800 to 2388)	13	10 (76.9%)	1953 ± 519 (1375 to 2884)
SMSC-1/P123	11	7 (63.6%)	1702 ± 450 (1321 to 2541)	12	10 (83.3%)	2010 ± 546 (1387 to 2979)

[a] The differences of the average ELISA titers and the percentage of positive samples between axmiDthe groups were not significant ($p>0.05$).
[b] SD was not determined because of minus values of some control titres.

Table 2: Serum antibody titers of chickens following infection with 3-1, 3-1/P60 and 3-1/ P123 isolates at 1-day of age

CAV isolate	16 days p.i.			30 days p.i.		
	No. of sera	No. of positive sera (%)[a]	Average titer ± SD (range)[a]	No. of sera	No. of positive sera (%)[a]	Average titer ± SD (range)[a]
Control	10	0 (0%)	32* (-129 to 332)	10	0 (0%)	62* (-98 to 337)
3-1	15	9 (60%)	1732 ± 492 (989 to 2489)	12	10 (83.3%)	2049 ± 523 (1431 to 2992)
3-1/P60	12	6 (50%)	1675 ± 440 (989 to 2244)	13	9 (69.2%)	1981 ± 554 (1387 to 2825)
3-1/P123	9	5 (55.5%)	1670 ± 416 (1259 to 2333)	12	9 (75%)	2001 ± 626 (1422 to 2924)

[a] The differences of the average ELISA titers and the percentage of positive samples between the groups were not significant ($p>0.05$).
[b] SD was not determined because of minus values of some control titres.

Table 3: Serum antibody titers of chickens following inoculation with BL-5 isolate at 1-day of age

CAV isolate	16 days p.i.		
	No. of sera	No. of positive sera (%)[a]	Average titer ± SD (range)[a]
Control	10	0 (0%)	39* (-120 to 332)
BL-5	10	7 (70%)	2603 ± 2063 (1047 to 7653)

[a] The differences of the average ELISA titers and the percentage of positive samples between the groups were not significant ($p>0.05$).
[b] SD was not determined because of minus values of some control titres.

of cell culture passage might contain different virus concentrations which were not titrated. Alternatively, the low and high passaged viruses may have undergone some form of adaptation in cell culture that limits them to replicate in chickens. Studies have shown that CAV that undergo high cell culture passages in MSB-1 are less pathogenic in susceptible chickens (Chowdhury *et al.*, 2003; Todd *et al.* 1995; 1998). It is not clear why the high number of cell passages should select a virus population that is less able to cause disease lesions in chickens. Todd *et al.* (1995) has speculated that adaptation of CAV to MSB-1 cells, which have been shown to possess the characteristics of mature T cells (Adair *et al.*, 1993), may make the virus less able to replicate in precursor erythroblastoid cells and as such is less effective in causing anemia.

This study provides information on the effectiveness of various passaged CAV isolates to produce antibody response. However, the low passaged CAV isolates, SMSC-1 and 3-1 were more pathogenic compared to the high passaged isolates, SMSC-1/P60, SMSC-1/P123, 3-1/P60 and 3-1/P123 viruses (Chowdhury *et al.*, 2003). It can be concluded that, the high passaged isolates may be suitable to be developed as candidates for live attenuated vaccines. However, the efficacy and safety of the high passaged isolates as live attenuated vaccine remains to be studied

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