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TOTAL HAEMOLYTIC COMPLEMENT PROFILE IN CHICKS FOLLOWING FOWL POX VACCINATION OR INFECTION

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Summary. — Total haemolytic complement levels were assessed in normal, fowl pox-vaccinated or infected chicks using radial immune haemolysis up to 28 days post-treatment. Significantly lower values of total haemolytic complement were recorded 7—21 days post-vaccination or 21 days post-infection as compared to controls ($p < 0.05$). The differences between intervals, the influence of the period of treatment were also significant, but the vaccinated chicks did not differ significantly from the infected ones ($p < 0.05$). It is concluded that lower circulating levels of total haemolytic complement may be due to deposition of complement at the sites of virus replication.

Key words: Fowl pox virus; fowl pox vaccine, total haemolytic complement; chicks

Introduction

Fowl pox virus is responsible for formation of cutaneous lesions in unfeathered parts (contagious epithelioma) and also of mucosal lesions (*Avian diphteria*) (Aitken *et al.*, 1977). The role of both humoral and cellular immune responses are now well established in the recovery of birds from this disease (Morita, 1973; Pathak *et al.*, 1974). Complement pathways have role to play in antiviral immune system (Hirsch, 1982).

The work on the poultry complement system has suffered due to lack of the availability of antisera to purified chicken complement components. The present studies were undertaken to assess the total haemolytic complement profile in fowlpox vaccinated or infected chicks as compared to control normal birds.

Materials and Methods

Chicks. Two hundred and fifty day old male chicks were obtained on complementary basis from Northern hatcheries, Ludhiana. They were provided with standard poultry management conditions and reared up to 7 weeks. Out of these hundred were finally randomly used for this experiment. Four experimental groups vaccinated, infected and two control groups (C_1 and C_2) were made. Each group consisted of twenty five birds. One control group each was raised in same poultry house with vaccinated and infected groups. All the birds were given similar poultry ration.

Vaccine. Six hundred doses of fowl pox vaccine (freeze-dried) (Batch No. Pox 7/84—85) were purchased from Punjab Veterinary Vaccine Institute, Ludhiana, Punjab. It is a live vaccine and prepared from the virus passaged on the chorioallantoic membrane of chick embryos. The vaccine was kept at —20 °C before use.

One vial of the vaccine was mixed in 30 ml of 50% glycerine-saline solution. The homogenized suspension was kept on ice during the course of its use. The reconstituted vaccine was used within one hour after its reconstitution.

Vaccination. Vaccination was done by the prick method into the wing web. Twenty five chicks were vaccinated.

Virus. The fowl pox virus (N.H./FZ/2214/27-8-85) was isolated from an outbreak of fowl pox in chicks brought to Pathology Laboratory, Northern hatcheries, Srabha Nagar, Ludhiana from Fazilka, Punjab by a pultry farmer. The virus was passaged twice on the chorioallantoic membrane (CAM) of chick embryos. The pock lesions and membrane thickening were visible. Red coloured elementary bodies were found by Gimenez staining (Tripathy and Hanson, 1976).

Ten per cent CAM suspension was used to infect the chicks in comb, wing web, and feather follicles.

Chicken serum. Blood samples were collected by cardial puncture, clotted for 15 min at room temperature and then left for half an hr at 37 °C and centrifuged. The samples were diluted two-fold in cold buffer and immediately used in the test.

Radial immune haemolysis test. The test was performed as described by Skeeles *et al.* (1979a). The final haemolysin concentration to be used for sheep red blood cell (SRBC) sensitization was determined by trying 1 : 50, 1 : 100, 1 : 200 and 1 : 400 haemolysin dilutions. The final haemolysin dilution of 1 : 100 in agarose gave the best results. The haemolytic radial diffusion plates were prepared as described by Gewurz and Suyehira (1976).The final mixture consisted of 6 ml agarose (2.5%), 4 ml of Gelatin-veronal buffer (GVB^+) and 1 ml of senzitized sheep red blood cells. The volume of SRBC to be incorporated was standard ized after a trial with 0.5, 1, and 2 ml of sensitized SRBC; the best results were obtained with 1 mı sensitized SRBC. The melted agarose and GVB^+ were maintained at 60 °C in a water bath. The tube was removed and finally sensitized SRBC were added and poured on the glass plate. It was kept in refrigerator for half an hr at 10 °C. Wells were punched by placing the immunoplate on a pattern of wells traced on a graph paper.

The serum samples were diluted two fold in GVB^+. A total of seven dilutions from 1 : 2 to 1 : 128 were made. The plates were loaded with serum dilutions and kept in humidity at 37 °C for 1 hr. Results could be easily read after incubation and were then kept overnight at 10 °C for further confirmation as lytic zones became more clear. Slides were stored for up to 3 months after staining with benzidine-acetic acid (Talwar, 1983). The reagents and buffers--GVB^+, GVB^+, $GGVB^{++}$ were prepared as described by Gewurz and Suyehra (1976). The final concentration of sensitized SRBC was adjusted to 1×10^9/ml using O.D. readings at 541 nm and total volume was adjusted accordingly. Briefly, 0.5 ml of cell suspension was lysed with 7 ml of distilled water. O.D. of V_1 volume at 541 nm/0.703 $\times$ $V_1 = V_2$. $V_2 - V_1$ = ml of 0.01 mol/l EDTA to be added to cell suspension V_1 and obtained the required SRBC concentration.

Biostatistical analysis was done using programme ANOVA. The simultaneously significant mean values were compared after calculating critical differences, as described (Lombard, 1975; Singh *et al.*, 1984). The complement titres in ratios to the geometric means were calculated.

Results and Discussion

Total haemolytic complement was assessed in chicks up to 28 days post--vaccination (p.v.) or post-infection (p.i.). Simultaneously equal number of control birds were also included in the study (Table 1). The reciprocal of the highest dilution of a sample showing a haemolytic ring was taken as the titre of total haemolytic complement.

The role of complement in vaccinia infection had been described long ago by Brier *et al.* (1971), who reported that cells infected with vaccinia virus

Table 1. Geometric mean of total haemolytic complement in fowl pox-vaccinated or -infected birds

Period (days) of treatment		0	7	14	21	28
	Age of birds (Weeks)	8	9	10	11	12
Vaccinated		2.0	2.4	5.7	3.6	3.6
Controls (C_1)		1.8	3.5	57	8.32	2.0
	Age of birds (Weeks)	7	8	9	10	11
Infected		1.4	2.0	3.6	6.9	4.4
Controls (C_2)		2.3	2.0	3.5	57	5.1

could be lysed by antibody in the presence of complement. The main obstacle in assessment of the role of complement in fowl pox virus infection was that the complement components of chicken have not yet been identified (Ohta *et al.*, 1983). Mono-specific Japanese quail C3 cross reacted with chicken C3 (Kai *et al.*, 1983). Ohta *et al.* (1983) used quail C3 antiserum and demonstrated antibody independent activation of chicken complement via alternative complement pathway by fowl pox virus. In the present studies, a peak of haemolytic complement (mean titre 57) was observed at age of 10 weeks in normal chicks; while the vaccinated and infected ones showed mean titres of 5.7 and 6.9, respectively, at this interval.

In the vaccinated group lower complement values were recorded at 7 and 21 days p.v. as compared to controls. But, on day 28 p.v. the mean total haemolytic complement levels were 3.6 as compared to 2.0 in the control C1 group. While in the infected group the lowest complement values were recorded on day 21 p.i. as compared to controls. The infected and vaccinated groups did not differ significantly in the haemolytic complement titres, as the vaccine was a live virus attenuated on CAM of chick embryos.

The differences between various age groups and the influence of treatments (vaccination or infection) at different age were significant by ANOVA ($P < 0.05$).

Ohta *et al.* (1983) reported deposition of C3 in progressive skin lessions from 6 days p.i. but at regression of lesions 14 days p.i. They also suggested that complement activation might participate on the formation of skin lesions by release of chemotactic factors such as C3a and C5a. Complement deposition is an immunopathological mechanism in fowl pox infection and decrease in circulating total complement levels be due to temporary depletion of serum of its complement components. This could also be due to injury to some site of production of complement.

Similar findings have been reported in infectious Bursal diseases (Skeeles *et al.*, 1979*b*). They have also reported immune complexes deposition at the sites of virus replication.

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