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MORTALITY AND ANTIBODY-FORMING-CELL RESPONSES FOLLOWING INOCULATIONS OF TURKEY AVIAN PARAMYXOVIRUS-TYPE 3 IN TWO BREEDS OF CHICKEN

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SUMMARY : An experimental infection with live Midland Poultry Holdings virus (MPHV) strain of turkey avian paramyxovirus-type 3 caused 100% (15/15) mortality among the Birmingham B2 chicks in 3-13 days after intravenous inoculation, compared to 53% (8/15) mortality among the White Leghorn chicks at 4-9 days post inoculation. The death response appeared to inversely correlate with the antibody-forming-cell (AFC) response to the inactivated MPHV as indicated by the higher AFC response (2.6 and 1.9 $\log_{10}$ anti-IgG (H+L) AFC per 10^7 splenocytes) in the WLH chicks inoculated with inactivated MPHV as compared to the lower AFC response (1.0 and <1.0 $\log_{10}$) in the similarly MPHV-inoculated B2 chicks.

Keywords: mortality, antibody-forming cells, Midland Poultry Holdings virus, turkey avian paramyxovirus-type 3, chicken, breed

INTRODUCTION

Russell *et al.* (1987) had converted an indirect immunoperoxidase (IIP) assay into an antibody-forming-cell (AFC) assay by replacing the antiviral antibody with splenocytes. The assay had been applied to investigate avian, murine or swine immune response by measuring B cell activity during immunisation with viral (Newcastle disease virus [NDV] and paramyxovirus [PMV]-3) or bacterial antigens.

In the present study, the AFC assay was used to compare the AFC responses in correlation with mortality to MPHV inoculations between two breeds of chicken, namely the Birmingham B2 and the White Leghorn.

MATERIALS AND METHODS

Virus

The seed MPHV, a strain of turkey avian paramyxovirus-type 3 was kindly provided by Dr. D.J. Alexander, the Central Veterinary Laboratory (CVL), Weybridge. It was propagated in allantoic cavities of 10-day-old embryonated hens' eggs. Harvested

infectious allantoic fluid was stored in small aliquots at -70°C, tested for bacterial contamination and titrated by an IIP test (Russell *et al.*, 1983), before being inoculated into the chicks.

In the present AFC assay, MPHV from the same virus stock was inactivated by 0.5% beta-propriolactone (BPL, Sigma) for two hours.

Specific pathogen-free chickens

Small breeding flocks of a selection of inbred chicken lines were kindly provided by Houghton Poultry Research Station. The Birmingham line was taken over by the station from Birmingham University in 1976. There were two sublines, homozygous for avian major histocompatibility complex (B-locus) alleles B2 and B21. B2 subline was used in this study.

The White Leghorn (WLH) chicks were obtained from the CVL.

The specific pathogen-free (SPF) chickens had been monitored for MPHV infection, Newcastle disease and pullorum disease. The flocks were maintained in indoor chicken runs and were fed and watered *ad libitum.*

Experiment on mortality

In the mortality experiment, 15 one-day-old chicks of each breed were each inoculated with 5.7 $\log_{10}$ IIP focus-forming units (ffu) of live MPHV in 0.1-ml volume via the jugular intravenous (IV) route. Similary, the control birds received the same volume of phosphate-buffered saline A (PBSA).

Experiment on AFC-response

In the experiment on AFC-response, two one-day-old chicks from each breed were each inoculated with 0.3 ml of the inactivated MPHV stock via the IV route. The control chicks received PBSA.

The procedure for AFC assay in the present study was based on the method of Russell *et al.* (1987). MDBK cells that had grown to confluence in circular semimicrowells (Costar) were coated with viral antigens by uniformly infecting them in each well with one ml of MEM medium containing 6.7 $\log_{10}$ ffu of MPHV, fixed for 10 minutes at room temperature with buffered 3.3% formol-saline, washed three times in tap water and stored as dry cells at -70°C.

At least one hour before the AFC assay, the antigen-coated wells were blocked with balanced salt solution (BSS) containing 5% newborn calf serum at 37°C.

Chicks were sacrificed by cervical dislocation. The spleens were immediately taken out and placed in BSS before being mashed with a syringe plunger onto taut nylon mesh (Fylitis nylon maille 124, Cadish and Sons, UK) fixed over a small plastic pot. The homogenised tissue was sieved through the cloth by flushing it with BSS from a syringe. The tissue suspension was allowed to sediment in plastic universal tubes for five minutes. The finer suspension above the sediments was washed twice in BSS by spinning at 1500 g for 10 minutes at room temperature. The pellet was resuspended in 1.0 ml of BSS. Ten-ml volumes of cell suspension were diluted at one in 10 in Natt and Herrick's (1951) stain that was allowed to set for five minutes before being counted in a haemocytometer.

In order to obtain optimal numbers of plaques for counting, suspensions of 10^7, 10^6, or 10^5 splenocytes in 1.0 ml of BSS were added to the wells, centrifuged at 1500 g for 10 minutes, and incubated at 39°C for two to three hours, free of vibration. The splenocytes

were then removed with three rinses of tap water and then replaced by 0.2 ml of horseradish-labelled conjugate, rabbit anti-chicken IgG (H+L) (Nordic Immunology, London) at 1: 1000 dilution in PBSA for direct AFC estimations. The conjugate was incubated at 37°C. After the 40-minute incubation, the similarly washed wells were replaced by 0.2 ml of substrate consisting of 0.006 % of H_2O_2 and orthodianisidine. AFC was revealed as brown precipitates as seen under 40-or 100-fold magnification using a Leitz Diavert inverted microscope. After colour development, the plates could be rinsed and stored dry for re-examination. Counts of AFC were converted to $\log_{10}$ and results were expressed as $\log_{10}$ AFC per 10^7 splenocytes. Counts of less than 1.0 $\log_{10}$ AFC per 10^7 splenocytes were considered as indicating no AFC response.

RESULTS

Mortality

All B2 chicks died on days 3 to 13 post inoculation (PI) with live MPHV, compared to only eight deaths out of 15 WLH chicks at days four to nine PI (Table 1).

Table 1. Mortality of B2 and WLH chicks following live MPHV infection

Treatment	Line	Death/ Group Size	% mortality	Death time (d)	
				range	mean
Virus + PBSA	B2	15/15	100	3-13	5.2
	WLH	8/15	53	4- 9	6.0
PBSA control	B2	0/15	0	Nil	Nil
	WLH	0/15	0	Nil	Nil

AFC Response

The AFC responses of chicks in the present experiments were measured by counting the number of AFC that were revealed as brown precipitates on the cell membrane. The anti-IgG AFC response between one-day-old WLH chicks and B2 chicks immunised with inactivated MPHV is shown in Table 2. Out of the initial 7.0 $\log_{10}$ splenocytes, each of the two WLH chicks had 2.6 and 1.9 $\log_{10}$ AFC, respectively, compared to 1.0 and <1.0 $\log_{10}$ AFC of each of the two B2 chicks. All non-immunised control birds of both breeds showed no AFC response.

DISCUSSION

The larger number of deaths in the infected B2 group than in the infected WLH group indicates that the chicks of the B2 breed were more susceptible than the chicks of

the WLH breed to live MPHV infection. With AFC assay using inactivated virus, interbreed influence on the pathogencity of the virus was evident (Table 2). With inactivated virus in the one-day-old birds, AFC response tended to be higher in the less susceptible breed such as WLH than the more susceptible B2 breed. These findings show that the mortality appears to be inversely correlated to the AFC response between the two breeds of chicken. This may imply that the inherent ability of the AFC to mount a humoral immune response to MPHV infection varies with different breeds of chicken.

The use of inactivated MPHV in the AFC assay most certainly indicates that the virus was sufficiently immunogenic but incapable of replicating in the chicks. The absence of morbidity or death following inoculation of the inactivated virus presumably shows that the virus was also non-toxic to the chicks.

Table 2. Comparative AFC response to inactivated MPHV between WLH and B2 chicks at seven days postinoculation

Treatment	Breed	Individual results (Log_{10} anti-IgG (H+L) AFC/10^7 splenocytes)
Inactivated MPHV	B2	1.0, <1.0
	WLH	2.6, 1.9
Control	B2	<1.0, <1.0
	WLH	<1.0, <1.0

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RINGKASAN

GERAK BALAS KEMATIAN DAN SEL PEMBENTUK ANTIBODI BERIKUTAN PENGINOKULATAN DENGAN PARAMIKSOVIRUS-TIP 3 AVIAN AYAM BELANDA DALAM DUA BAKA AYAM

Suatu jangkitan ujikaji dengan strain virus Midland Poultry Holdings (MPHV) dari paramyxovirus-tip 3 avian ayam belanda yang hidup menyebabkan kematian 100% (15/15) di kalangan anak ayam Birmingham B2 dalam 3-13 hari selepas penginokulatan intravena, berbanding dengan kematian 53% (8/15) di kalangan anak ayam White Leghorn (WLH) 4-9 hari selepas penginokulatan. Gerak balas kematian itu kelihatan berkorelasi songsang dengan gerak balas sel pembentuk antibody (AFC) terhadap MPHV yang telah ditakaktifkan, seperti yang ditunjukkan oleh gerak balas AFC yang lebih tinggi (2.6 dan 1.9 log_{10} anti-IgG (H+L) AFC per 10^7 splenosit) dalam anak ayam WLH yang telah diinokulat dengan MPHV takaktif berbanding dengan gerak balas AFC yang lebih rendah (1.0 dan <1.0 log_{10}) dalam anak ayam B2 yang juga telah diinokulat dengan MPHV takaktif.