

CELL-MEDIATED IMMUNE RESPONSE TO EGG DROP SYNDROME-76 (EDS-76) VIRUS INFECTION IN CHICKENS

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ABSTRACT

Chicks infected with 320 HA units of EDS-76 virus (virus-127) by the intraocular route showed significant cell-mediated immune response by the second week after infection. The response persisted till the fifth week. Reinfection after the ninth week with 320 HA units of EDS-76 virus (virus-127) did not result in any significant response, as measured by lymphocyte stimulation test and leucocyte migration inhibition test.

INTRODUCTION

McFERRAN *et al*¹ reported a new adenovirus infection in chicken in Ireland which caused a drastic drop in egg production. The disease was named egg drop syndrome-76 (EDS-76) and subsequently the disease was reported in Italy², United States³, Belgium⁴, Israel⁵, West Germany⁶, Singapore⁷, Australia⁸, Japan⁹, Iraq¹⁰ and Greece¹¹. There is serological and clinical evidence of this infection in India^{12,13}. Though there are many reports on the antibody response to EDS-76 infection, there is no report of evidence for the role of cell-mediated immunity (CMI) in EDS-76 infection. This report gives the results of studies on cell-mediated immune response in chicks experimentally infected with EDS-76 virus (virus-127) using the lymphocyte stimulation test (LST)¹⁴ and the leucocyte migration inhibition test (LMIT)¹⁵.

MATERIALS AND METHODS

Experimental chicks were obtained from Balaji Hatcheries, Chittoor. Tritiated thymidine (³H]-thymidine) was supplied by the Isotope Division, Bhabha Atomic Research Centre, Bombay. EDS-76 virus was obtained from Dr J. B. McFerran, Veterinary Research Laboratory, Starmont, Belfast, Northern Ireland. All LST samples were counted in a Beckman LS-150 liquid scintillation counter. Non-heparinized capillaries for LMIT were supplied by Top Syringe Manufacturing Co., Bombay, and leucocyte migration chambers were obtained from Laxbro, Pune. RPMI-1640 medium, purchased from Hi-media, Bombay, was used for both LMIT and LST.

Experimental design

Twenty chicks out of forty were infected in four groups with 320 HA units of EDS-76 virus (virus 127) by i/ocular route. Reinfection was done after the ninth week to study anamnestic response. Other twenty chicks were kept as controls.

Lymphocyte stimulation test

Lymphocytes from the peripheral blood of the infected and control chicks were purified by Boyum's technique and lymphocyte cultures were set up¹⁶ by dispensing 0.5 ml of cell suspension containing 1×10^6 viable cells into each tube. They were incubated with or without 256 HA units of EDS-76 virus (virus-127) in RPMI-1640 medium at 37°C in a humid chamber under 5-10% carbon dioxide tension for four days. Approximately 18-20 h before the end of the incubation period 1 μ Ci of [³H]thymidine in 10 μ l of medium was added to each culture. After four more days of incubation 10 ml of cold normal saline was added to each culture tube and the contents were filtered through 0.22 μ m Millipore filters. The filters were then washed with 10 ml of 5% trichloroacetic acid followed by 10 ml of absolute methanol. The filters were removed and dried in scintillation vials and 10 ml of dioxane-based scintillation fluid was added to each vial. Counts (cpm) were taken in a liquid scintillation counter. Stimulation index (SI) was calculated by using the formula

$$SI = \frac{\text{Mean cpm with antigen}}{\text{Mean cpm without antigen}}$$

Leucocyte migration inhibition test

This was carried out¹⁵ in leucocyte migration

chambers. After separating the leucocytes by low speed centrifugation¹⁷, they were suspended in 0.4 ml of RPMI-1640 medium. Capillaries were then filled with the leucocyte suspension. One end of the capillaries was sealed with semisoft paraffin wax. The sealed capillaries were placed in a test tube and centrifuged at 120 *g* for 1–2 min. The capillaries were then removed and cut at the cell–liquid boundary. The cut pieces containing packed leucocytes were fixed in migration chambers using semisoft paraffin wax. The chambers were slowly filled with RPMI-1640 medium and covered with coverslips. Three chambers were kept with medium containing 256 HA units of EDS-76 virus (virus-127) per ml. Three other chambers were kept as controls without addition of antigen. The chambers were placed in a moist container and incubated at 37°C under 5–10% carbon dioxide tension for 18–20 h. Migration zone was recorded using a camera lucida and then the area of the migration zone was measured with planimeter. Per cent area of migration was calculated¹⁸ using the formula

$$\frac{\text{Average area of migration of cells with antigen}}{\text{Average area of migration of cells without antigen}} \times 100.$$

RESULTS AND DISCUSSION

The results of LMIT and LST are presented in table 1. The stimulation indices (SI) were expressed

as logarithms to overcome the problems of non-homogeneity of variance. Student's *t* test was used for the statistical analysis. Significant SI was observed from the 2nd to the 4th week, with maximum SI appearing at the 3rd week after infection. From the 5th to 7th weeks the difference between SI values of control and infected chicks was not significant. LST was not done on the 8th and 9th week samples. Reinfection after the 9th week did not result in significantly higher SI values till the 12th week, when the last sample was taken.

One week after the infection the per cent area of migration in LMIT for infected chicks was not significantly different from that for control chicks. Significant LMI response was observed at the 2nd week after infection and lasted till the 5th week. From the 6th week to the 9th week the LMI response was not significant. Reinfection after the 9th week did not result in significantly different mean per cent area of migration in infected chicks.

The results indicate that significant CMI started appearing by the 2nd week and persisted only for 4 weeks, i.e. up to the 5th week after infection. Reinfection after the 9th week did not produce significant CMI response during 5 weeks after reinfection.

There are no other reports on the use of LST and LMIT in EDS-76 infection with which to compare the results of the present study. The present results show that LMIT detected the infection for a longer time than LST. This may be due to the use of a higher concentration of cells in LMIT than in LST.

Table 1 LMI and LS responses of chicks infected with EDS-76 virus (virus-127)

Collection (weeks)	LMI response (mean per cent area of migration)		LS response (log SI)	
	Control chicks	Infected chicks	Control chicks	Infected chicks
1	99.3 ± 3.16	91.9 ± 2.19	0.0649 ± 0.032	0.2732 ± 0.018
2	103.1 ± 2.83	67.0 ± 5.61†	0.0573 ± 0.023	0.5798 ± 0.036†
3	105.5 ± 4.07	61.8 ± 1.19††	–0.0422 ± 0.014	0.6599 ± 0.021†
4	100.3 ± 1.69	68.9 ± 3.06††	0.0656 ± 0.027	0.5403 ± 0.063†
5	104.8 ± 6.87	72.62 ± 4.83†	0.0864 ± 0.036	0.2306 ± 0.054
6	98.7 ± 4.61	85.7 ± 1.91	0.0531 ± 0.021	0.2405 ± 0.016
7	102.6 ± 2.98	90.0 ± 2.11	0.0607 ± 0.0308	0.2041 ± 0.027
8	99.92 ± 5.61	94.75 ± 4.07	ND	ND
*9	101.1 ± 2.67	97.2 ± 2.12	ND	ND
10	100.1 ± 5.68	83.0 ± 1.53	–0.0535 ± 0.014	0.1931 ± 0.028
11	102.3 ± 4.31	89.3 ± 2.94	0.0618 ± 0.025	0.2014 ± 0.063
12	99.81 ± 3.63	90.9 ± 1.81	0.0611 ± 0.0208	0.1990 ± 0.043
13	101.5 ± 2.72	91.6 ± 3.14	ND	ND
14	103.2 ± 6.38	96.7 ± 2.61	ND	ND

Each value is the average of 4 results; *Reinfection with 320 HA units of EDS-76 virus; Significance of difference from control values: †*P* < 0.05, ††*P* < 0.01; ND, Not done.

Further, in LMIT, in addition to lymphocytes, mononuclear cells and granulocytes are also involved, whereas in LST only purified lymphocytes are involved. Similarly it was reported that LMIT was more sensitive than LST in detecting CMI response to avian tuberculosis¹⁹. LMIT has the advantage that it is simple and economical and can be performed in any laboratory with minimum facilities; LST involves the use of radioisotopes and expensive liquid scintillation counters which may not be available in many laboratories. The poor CMI after reinfection may be probably due to immunosuppression brought about by the primary infection. The fact that leucocytes could be utilized as infective material for EDS-76 virus¹² and the isolation of EDS-76 virus from the leucocytes²⁰ of affected birds support this.

Thus *in vitro* CMI techniques standardized for EDS-76 can be used for laboratory confirmation of the diagnosis as well to monitor response to vaccines. Further studies are in progress.

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