

Cell Mediated Immunity to Avian Coccidiosis Following Administration of Experimental Vaccine(S)

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Abstract

Cell mediated immune response in chickens vaccinated with experimental vaccine against coccidiosis was detected by modified splenic cell migration inhibition test. The migration of the splenic cells from chickens immunized with Vaccine I (supernatant from sonicated sporulated oocysts) and Vaccine II (sediment from sonicated sporulated oocysts) were inhibited remarkable with antigen and there was a significant difference ($P<0.05$) in migration distance of the splenic cells with and without antigen. The mean migration index was 47.5 and 64.1 per cent, respectively. The migration of the splenic cells from immunized chickens with Vaccine III (unsonicated sporulated oocysts) and control group (injected with PBS) was not inhibited with antigen; although there was a significant difference ($P<0.05$) in migration distance of the splenic cells with and without antigen. The mean migration index was 74.0, 83.9 per cent, respectively. Results of the challenge experiments revealed that the Vaccine-I gave maximum protection (>80 %) followed by Vaccine II (60 %) and Vaccine III (30 %). Results of the MIF and challenge responses indicate that the Vaccine-I induced a strong cell mediated immune response as immune chicks resisted the heavy dose of challenge and gave maximum protection.

Key words: Coccidiosis, cell mediated immune response, experimental vaccine

Introduction

Avian coccidiosis is a complex protozoan disease that continues to plague the poultry industry worldwide (Soulsby, 1982). In Pakistan, million of rupees are spent every year on traditional prophylactic chemotherapy and continuous to be the most widely preferred mode of treatment.

Unfortunately the rapid emergence of drug resistant parasitic strains, increased developmental costs associated with new anticoccidial drugs, and the possible future restraints by medication of feed in animals used for human consumption have prompted renewed interest in alternative methods to control coccidiosis, which is through vaccination.

Various attempts have been made on the development of vaccine against coccidiosis in different parts of the world. For this purpose several methods were studied such as oocyst administration at low and controlled doses (Caron *et al.*, 1997), administration of the strains that had been attenuated by physical or biochemical means (Yvori and Naciri 1986), immunization with extracted or recombinant antigens (Anonymous, 1996). Work has also been done in the use of hybridoma technique to produce monoclonal antibodies and its application in the immunological control of coccidiosis (Davis *et al.*, 1979). The inoculation of antibodies *per se* does not seem to be a viable procedure.

Commercial vaccines like "Immunocox" and "Coxivac" are available in some parts of the world to control the coccidiosis. Immunocox is also available in Pakistan but does not provide 100 per cent protection and the disease occurs inspite of vaccination (Shaker, 1997). In our previous studies an experimental vaccine from local isolates of coccidian was prepared and evaluated on the basis of humoral and challenge responses (Akhtar *et al.*, 2001).

Present paper reports the cell mediated immune response against avian coccidiosis following experimental vaccine(s) prepared from local isolates of genus *Eimeria*.

Materials and Methods

Collection and Sporulation of oocyst

Oocyst (mixed species of genus *Eimeria*) recovered from the naturally infected chickens were sporulated (Akhtar *et al.*, 1998). The oocysts per mL count were done by McMaster counting technique (Hayat and Akhtar, 2000).

Preparation of sonicated antigen and vaccines

Sporulated oocysts were given 3-4 washings with phosphate buffered saline (PBS; pH 7.2). A

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concentration of 4,000 oocysts per mL was maintained with PBS. These were stirred continuously on a magnetic stirrer for twelve hours and then subjected to ultra-sonication (Akhtar *et al.*, 1998). Centrifuged at 2000 rpm for five minutes, supernatant and sediment as antigen(s) were collected separately for vaccine(s) preparation.

Following vaccines were prepared by inactivating with formalin (Akhtar *et al.*, 2001).

Vaccine-I: contain supernatant from sonicated sporulated oocyst

Vaccine-II: contain sediment from sonicated sporulated oocyst

Vaccine-III: contain un-sonicated sporulated oocyst

All the vaccines were stored in refrigerator.

Experimental design

One hundred day old broiler chicks were purchased from the local market were reared under standard managemental conditions in the experimental station Department of Veterinary Parasitology, University of Agriculture, Faisalabad, Pakistan. The birds were fed commercial ration. Fresh and clean water was given through out the experiment. Chicks on day 6 were equally divided into four groups. The detail for each group is as under:

Group-I Given Vaccine I @ 0.25ml per chick, orally

Group-II Given Vaccine II @ 0.25ml per chick, orally

Group-III Given Vaccine III @ 0.25ml per chick, orally

Group-IV Given PBS @ 0.25ml per chick, orally

On day 15 post vaccination, 15 birds from each group were slaughtered to collect the spleens.

Cellular immune response

Spleens were minced separately into 0.5 mm fragments in sterilized petri plates containing enough volume of Hank's Balanced salts solution (Flow Lab., UK). Splenic cell migration inhibition test following the method of Morita *et al.*, (1973) with modifications as described by Akhtar *et al.* (1999) was used to detect migration inhibition factor (MIF).

Challenge experiment

On day 15 post- vaccination, all the remaining chicks in group I, II, III and IV

(10 chicks in each group) were given oral dose of 60,000-70,000 sporulated oocysts of mixed species of genus *Eimeria*. Their faecal sample were collected daily up to day 35 post-vaccination. Number of oocysts per gram of droppings were calculated from each group.

Mortality occurring in all the experimental and control groups during the study period and autopsical findings were recorded. The intestines and caeca of birds died during the experiments and those of slaughtered at the end were examined to assess the severity of the disease.

Results and Discussion

A planned immunization programme for the control of coccidiosis in chickens is commercially available for quite some time (Edgar, 1958), marketed under the name CocciVac. The poor feed conversions which may initially result after vaccination, along with a several-week-period required for solid immunity to develop, make this vaccine less desirable (Gaimborne *et al.*, 1980). Immunocox available in the market has not given promising results against coccidiosis in Pakistan (Shaker, 1997). In our previous studies, polyvalent inactivated vaccine (s) against avian Coccidiosis from local isolates of coccidia was prepared and their humoral immune response was detected (Akhtar *et al.*, 2002).

It is documented in the literature that cell mediated immunity plays a major role in resistance to infection by coccidia. T-lymphocytes appear to respond to coccidial infection through both cytokine production and direct cytotoxic attack on infected cells (Lillehoj and Trout, 1996). In the present studies cell mediated immune response was detected using splenic cell migration inhibition test.

The migration of the splenic cells from chickens immunized with Vaccine I and Vaccine II were inhibited remarkable with antigen and there was a significant difference ($P < 0.05$) in migration distance of the splenic cells with and with out antigen. The mean migration index for vaccine-I and vaccine-II was 47.5 and 64.1 per cent, respectively.

Table 1: Mean migration distance and migration index.

Vaccinated Groups	Average distance (μ m)		Migration index (%)
	With antigen	Without Antigen	
Group I	328.80	691.30	47.50
Group II	400.00	617.00	64.10
Group III	709.00	958.00	74.00
Group IV	759.30	910.30	83.90

The migration of the splenic cells from immunized chickens with Vaccine III and control group (injected with PBS) were not inhibited with antigen; although there was a significant difference ($P < 0.05$) in migration distance of the splenic cells with and without antigen. The mean migration index for vaccine-III and control group was 74.0, 83.9 per cent, respectively.

The migration of sensitized splenic T-cells inhibited more with antigen is due to the fact that these sensitized T-cells are re-sensitized with the test antigen *in vitro*. Sensitized splenic T_{dth} cells release interleukine-I, interleukine-2, interleukine-4 and other cytokines including migration inhibition factor (MIF) which inhibit the migration of macrophages (Barrett, 1988). In the present studies, the migration of the splenic cell is inhibited by the MIF which indicate that the test antigen has triggered the T-cells, to initiate the cell mediated

immune response. In most parasitic infections, protection can be conferred experimentally on normal chicks by the transfer of spleen cells, especially T cells, from the immune chicks. This is because that these T-cells secrete interleukine-10 which inhibits the production and activity of the interferon-gamma required to activate macrophages and eliminate the parasitic infection (Roitt *et al.*, 1998).

Results of the challenge experiments revealed that the Vaccine-I gave maximum protection (>80 %) followed by Vaccine II (60 %) and Vaccine III (30 per cent) to the challenge.

Birds in group I were alert, active and healthy. Water and feed intake was normal. No mortality was recorded in this group after challenge. Oocyst appeared in the faeces on day 10 post challenge (25 days age) showing 150-250 oocyst per gram of faeces which gradually increased to 650-1000 EPG on day 16 (31 days age) post challenge. Oocysts number per gram of faeces decreased to 300-450 on day 20 (35 days age) post challenge.

Chicks in group II were partially normal. Some chicks were found lazy, huddling together in the corner, tired, exhausted, drooping of wings and consume less feed and water as compared to the control group. Eight birds from this group died after challenge. Oocyst appeared in the faeces on day 8 post challenge (23 days age) showing 4,00,000-5,50,000 oocyst per gram of faeces which gradually increased to 750,000-10,00,000 EPG on day 16 (31days age) post challenge. Oocyst number per gram of faeces decreased to 150,000-260,000 on day 20 (35 days age) post challenge.

Chicks in group III were not normal. Most of the birds were diseased and showing tiredness, laziness,

exhausted and unthriftiness. They were dull, depress huddle together in corners with the bloody diarrhoea. Oocyst appeared in the faeces on day 7 post challenge (23 days age) showing 9,00,000-9,50,000 oocyst per gram of faeces which gradually increased to 12,000,000-12,08,000 EPG on day 16 (31days age) post challenge. Oocyst number per gram of faeces decreased to 8,60,000-9,50,000 on day 20 (35 days age) post challenge It gave only 30% protection.

On day 5 post challenge, change in the behavior of the birds of group IV was observed. The birds were found uninterested in feeding but were drinking water normally. They were dull, depressed and were looking tired. They huddle together in the corners with drooping of wings and looking very much exhausted. Characteristic bloody diarrhoea was observed in all the birds of this group. All the birds died due to coccidiosis at different intervals. Oocyst appeared in the faeces on day 5 post challenge (20 days age) showing 10,60,000-13,50,000 oocyst per gram of faeces which gradually increased to 16,00,000-18,50,000 EPG on day 16 (31days age) post challenge. Oocyst number per gram of faeces decreased to 11, 00,000-14, 05,000 on day 20 (35 days age) post challenge.

Results of the MIF and challenge responses indicate that the Vaccine-I induced a strong cell mediated immune response as immune chicks resisted the heavy dose of challenge and gave maximum protection. The resistance to infection due to vaccine against coccidiosis may be due to the specific cytokines production and direct cytotoxic attack on infected cell (Lillehoj and Trout 1993, 1996; Rothwell, *et al.*, 1995; Dunn *et al.*, 1995).

Table 2: Oocysts count per gram of faeces recorded from vaccinated and control groups.

Days post infection	Groups (OPG)			
	Group I	Group II	Group III	Group IV
15	-	-	-	-
20	-	-	-	1060000-1350000
22	-	-	900000-950000	-
23	-	400000-550000	-	-
25	150-250	-	-	-
31	650-1000	750000-1000000	12000000-1108000	1600000-1800000
35	300-450	150000-260000	860000-950000	1100000-1405000

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