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Delayed Hypersensitivity Reaction as a Measure of Cell Mediated Immunity in Chickens Vaccinated with Sonicated Coccidial Vaccine

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Abstract: Delayed hypersensitivity reaction was used to measure the cell mediated immunity against avian coccidiosis. There was a gradual increase in the comb thickness of the immunized birds of all the groups from 24 to 72 hours after intradermal injection. The mean difference in comb thickness before and after injection of the respective antigen for the group immunized with Vaccine I (sonicated supernatant) was 1.87 mm at 24 hours, 2.23 mm at 36 hours, 2.31 mm at 48 hours and 2.42 mm at 72 hours. There was a significant difference ($p<0.01$) in comb thickness at 24 and 36 hours after intradermal injection. The mean difference in comb thickness before and after injection of the respective antigen for the group immunized with Vaccine II (sonicated sediment) was 1.12 mm at 24 hours, 1.75 mm at 36 hours, 2.10 mm at 48 hours and 2.31 mm at 72 hours. The difference was significant ($p<0.01$) at 24 and 48 hours; 36 and 72 hours after injection. Maximum difference in comb thickness was recorded 72 hours after injection in birds immunized with Vaccine I. It appears that delayed hypersensitivity response to sonicated supernatant antigen reported herein represent a strong CMI reaction. Delayed hypersensitivity comb reaction is a quick, simple, economical and practical tool to routinely determine the immune status of a bird with out restoring to challenge.

Key words: Delayed hypersensitivity reaction, cell mediated immunity, chickens

Introduction

Coccidial infections in chickens induce a variety of immune responses, many of which have been reported to adversely affect the parasite (Rose, 1976). Cell mediated immunity (CMI) has been shown to play a major role in resistance to coccidia In other animals (Klesius *et al*., 1975, 1976; Liburd *et al*., 1972), little published work in this regard is available for chickens (Morita *et al*., 1973; Giambrone *et al*., 1980; Akhtar *et al*., 1999). Different methods have been used to detect the CMI in avian coccidiosis with variable success. In the present study, chicks immunized with sonicated coccidial vaccine(s) were examined for their ability to develop significant delayed hypersensitivity reactions as a measure of CM.

Materials and Methods

Preparation of sonicated antigen: Sonicated antigen (s) from sporulated oocysts was prepared following the method of Akhtar *et al*. (1999). Briefly, concentrated suspension of sporulated oocysts (4000/mL) was stirred on a magnetic stirrer continuously for 12 hours and then subjected to ultra sonication for 1x10 minutes. The sonicated material was centrifuged mom rpm/30 minutes/4°C). Supernatant and sediment were collected separately to use as antigen and stored at 4°C.

Preparation of Vaccines: Following vaccines were prepared from the sonicated antigen (Akhtar *et al*., 1999).

Vaccine I	supernatant from sonicated sporulated oocysts
Vaccine II	sedimented from sonicated sporulated oocysts

The vaccines were stored at 4°C until use.

Experimental Designs: Sixty day old broiler chicks purchased from the local market were reared under standard managementsl conditions in the Experimental Station, Department of Veterinary Parasitology, University of Agriculture, Faisalabad. Day after arrival all the birds were given Newcastle Disease Virus vaccine. On day six, chicks were divided into three groups, having 20 chicks in each group. Group I, Group II were given Vaccine I and Vaccine II, respectively. The vaccines were given orally at 0.25 ml per chick. Group III was given phosphate buffered saline (PBS) at 0.25 ml per chick.

Delayed Hypersensitivity comb test: Delayed hyper sensitivity test following the method of Giambrone *et al*. (1980) with certain modifications was carried out to detect the cell mediated immune response. For this purpose, each group of immunized chicks were given the following allocated antigen (16 days post vaccination) intradermally (0.1 mL) in the comb with a 25 gauge needle. Group I was injected supernatant sonicated antigen, Group II was injected sediment sonicated antigen, Group III was injected PBS.

Thickness of the comb of individual chick was measured with vernier caliper before the inoculation of the antigen into the comb at 24, 36, 48 and 72 hours post injection. The results were analysed statistically.

Results and Discussion

Cell mediated immunity (CMI) expressed as delayed hypersensitivity can readily determined by intradermal skin test in many species except the chickens (Klesius *et al*., 1977a). Several studies have indicated that the wattle can be used to measure this type of CMI reactivity in a manner analogous to that of the skin test (Cooper *et al*., 1966; Warner *et al*., 1971; Morita and Soekawa, 1972). In the present studies, delayed comb hypersensitivity reaction was used to test its validity as a measure of cell mediated immunity against avian coccidiosis There was a gradual increase in the comb thickness of the immunized birds of the groups I and II from 24 to 72 hours after intradermal injection (Table 1).

The mean difference in comb thickness before and after injection of the respective antigen for the group immunized with Vaccine I was 1.87 mm at 24 hours, 2.23 mm at 36 hours. 2.31 mm at 48 hours and 2.42 mm at 72 hours. There was a significant difference ($p<0.01$) in comb thickness at 24

and 36 hours after intradermal injection, however the difference was non significant at 48 and 72 hours after injection. Among 20 immunized birds, comb thickness difference before and after injection ranged from 1.0 mm to 2.2 mm, 1.9 to 2.3 mm, 2.0 to 2.5 mm, 2.1 to 3.1 mm at 24, 36, 48 and 72 hours post injection, respectively.

Table 1: Delayed comb reactions in immunized chicks

Group	Mean* thickness difference in mm			
	24 hours	36 hours	48 hours	72 hours
GroupI	1.87	2.23	2.31	2.42
Group II	1.12	1.75	2.10	2.31
Group III	0.15	0.20	0.32	0.37

*Mean of 20 chicks

The mean difference in comb thickness before and after injection of the respective antigen for the group immunized with Vaccine II was 1.12 mm at 24 hours, 1.75 mm at 36 hours, 2.10 mm at 48 hours and 2.31 mm at 72 hours. There was a non significant difference in comb thickness at 24 and 36 hours; 48 and 72 hours after intradermal injection, however the difference was significant ($p<0.01$) at 24 and 48 hours; 36 and 72 hours after injection. Among 20 immunized birds, comb thickness difference before and after injection ranged from 0.8 mm to 1.5 mm, 1.3 to 2.0 mm, 1.3 to 2.2 mm, 1.9 to 2.8 mm at 24, 36, 48 and 72 hours after injection, respectively.

Delayed hypersensitivity reaction develop when antigen activates sensitized TDTH cells, these cells generally appear to be a TH1(CD4 T cells) subpopulation resulting in the secretion of various cytokines including 1L-2, gamma interferon, macrophage migration inhibition factor and tumour necrosis factor beta. The overall effect of these cytokines is to draw macrophage into the area and activate them. These reaction typically take 48-72 hours to develop (Roitt *et al*., 1998).

For the interpretation of CMI reaction, only the 72 hour reactions were included in the present studies since at this time the largest difference was recorded before and after intradermal injection. Using a difference of 0.7 mm before and after intradermal injection as a positive reaction (Klesius *et al*., 1977b). It was determined that immunized chicks of all the groups gave positive hypersensitivity reaction except the control group injected with PBS. in control group the difference in comb thickness before and after intradermal injection was non significant and was less than 0.7 mm. The difference was maximum 72 hours after injection in birds immunized with Vaccine I. It appears that delayed hypersensitivity response to sonicated supernatant antigen reported herein represent a strong CMI reaction (Giambrone *et al*., 1980).

From the results, it could be concluded that delayed hypersensitivity comb reaction is a quick, simple, economical and practical tool to routinely determined the immune status of a bird with out restoring to challenge.

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