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Modified Splenic Cells Migration Inhibition Test For the Detection of Cell Mediated Immunity Against Coccidiosis in Chickens

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bstract

A splenic cell migration inhibition test with modifications was successfully employed in the flat bottom micro titration plates to detect the cell mediated immunity more conveniently and accurately due to vaccine against coccidiosis. A drop of melted agarose (0.2% in PBS) was used to immobilize the splenic fragments. The splenic cells were culture in Hank's Balanced salt containing fresh chicken plasma and maintained for 10 days. Twenty four hours after cultivation, dominant cell type migrated from a splenic fragment was leukocyte-like cells. At the fourth day these appeared predominantly large round cells nd resembled to the macrophage. At the 10th day, spindle cells resembling of fibroblast appeared. The migration of the plenic cells from immunized chickens were inhibited remarkably with the antigen and there was a significant difference P<0.01) in migration distance of the splenic cells with and without the antigen.

troduction

layed hypersensitivity and splenic cell migration inhibition ve been frequently employed to detect cellular response ainst coccidiosis (Kelsus *et al.*, 1977; Morita *et al.*, 973). The earlier test is well characterized and classified ethod to detect cellular response in vaccinated birds. owever, the injection of antigens into an animal may alter immunological status and further evaluation of humoral cellular responses may not be valid (Jones and Buening, 83). Therefore, certain *in vitro* assays have been widely ed for detecting specific lymphocyte reactivity in birds thout altering the immunological status. Splenic cell gration inhibition is a sensitive and successfully used hnique to assay cellular response. The limitation in the sting technique is that the migration distance cannot be asured accurately due to the constraints in the mobilization of the splenic fragments in the petri dishes esent study reports the modification in the splenic cell ration inhibition test to make it more convenient and urate.

terials And Methods

ction and Sporulation of oocyst: Oocyst (mixed cies of coccidia) recovered from the naturally infected kens with coccidia were sporulated (Akhtar *et al.*, 8). The oocyst per mL count was done by McMaster ting technique (Gorden and Whitlock, 1939).

ation of sonicated antigen: Sporulated oocysts were 3-4 washings with phosphate buffered saline (PBS; 7.2) and a concentration of 4,000 per mL was ained with PBS. These were stirred continuously on a etic stirrer for twelve hours and then subjected to nication (Akhtar *et al.*, 1998). The suspension was uged (6000 rpm/30 minutes/4°C). Supernatant thus ted was used as an antigen for vaccine preparation r *et al.*, 1998).

Experimental Design: Ten day old broiler chicks were procured from the local market. Chicks were reared under the standard managemental conditions at the Department of Veterinary Parasitology, University of Agriculture, Faisalabad. Chicks at the days 5 were divided into two groups viz. A and B having five birds in each group. Group A was given vaccine (Akhtar *et al.*, 1998) orally (0.25 mL per chick) and group B was kept as control and was given PBS (0.25 mL per chick).

Spleens were removed from the immunized and unimmunized (control) chickens 7 days after the vaccination and minced separately into about 0.5 mm fragments in a sterilized petri plates containing enough volume of Hank's Balanced Salt Solution (Flow Lab., UK). From the control birds blood was collected to separate plasma.

Test Procedure: Splenic cell migration inhibition test following the method of Morita *et al.* (1973) with modifications was performed. Flat bottom micro titration plates (Flow Lab. UK) were used. One small size drop of 0.2 per cent melted Agarose (Sigma Chemical Co. USA) prepared in double distilled water was dispensed into the middle of each well of micro titration plate (96 wells) with a Pasteur's pipette.

One fragment of spleen was placed in each well with the help of a fine forceps and pressed gently so that the splenic fragment adhere to the agar. A 50 uL of fresh plasma collected from control birds was added to all the wells with a twelve-channel micropipette. A 250 uL of HBSS was dispensed in each well. A 50 uL of the antigen was added in all wells of the alternative rows. Plates were covered and incubated at 37°C for 24 hours. Cellular migration from the edge of the fragment was measured by an ocular micrometer under the inverted microscope (Nikon, Japan). The culture was maintained for 10 days and observations were recorded.

Mean migration distance was calculated from the migration

distances of 10 fragments. A migration index was calculated by the following formula:

$$\frac{\text{Mean migration distance in the presence of the antigen}}{\text{Mean migration distance in the absence of without the antigen}} \times 100$$

Birds in each group were challenged with 30,000 sporulated oocyst (mixed species) at days 10 post vaccination. Fecal examination was conducted daily and number of oocyst per gram of droppings were determined up to 20 days. Clinical symptoms were observed daily in each group.

Results and Discussion

A splenic cell migration inhibition test in several animals including poultry has been described as an *in vitro* indicator of the cell mediated immunity (Carpenter, 1963; Morita and Soekawa, 1972). Although the test has been used successfully to detect the cell mediated immunity in chicken against coccidiosis (Morita *et al.*, 1973) but there are certain limitations in the existing technique. In the present paper, the test has been modified to make it more convenient and accurate. Previously, the test was performed in petri dishes and the migration distance was not accurately measured because the splenic fragments cannot be immobilized. Further large quantity of the cell culture medium was required to maintain the cells in the petri plates (Mortia and Soekawa, 1972) in 5 per cent CO_2 incubator. In the present studies, the test was performed in flat bottom micro titration plates where the splenic fragments were immobilized by placing a drop of 0.2 per cent Agarose and the migration distance was accurately measured. Twenty four hours after cultivation, dominant cell type migrated from splenic fragment was leukocyte-like cells. At the fourth day these appeared predominantly large round cells and resembled to the macrophage. At the 10th day after cultivation, spindle cells resembling of fibroblast appeared. As shown in the Table 1, the migration of the splenic cells from immunized chickens were inhibited remarkably with antigen and there was a significant difference ($P<0.01$) in migration distance of the splenic cells with and with out antigen. 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 Tdth 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 animals by the transfer of spleen cells, especially T cells, from the immune animals. This is because that these T-cells secrete interleukine-4 a interleukine-10 which inhibit the production and activity the interferon-gamma required to activate macrophages eliminate the parasitic infection (Roitt *et al.*, 1998).

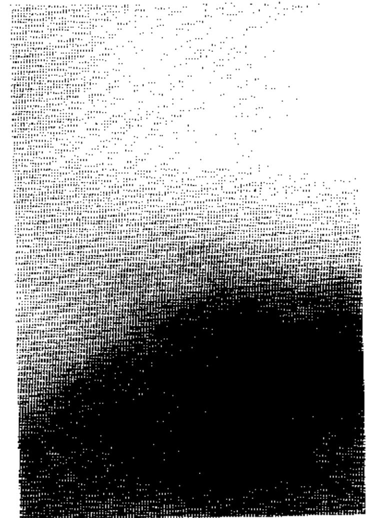

Fig. 1: Migration of the spleenic T-cells with antigen

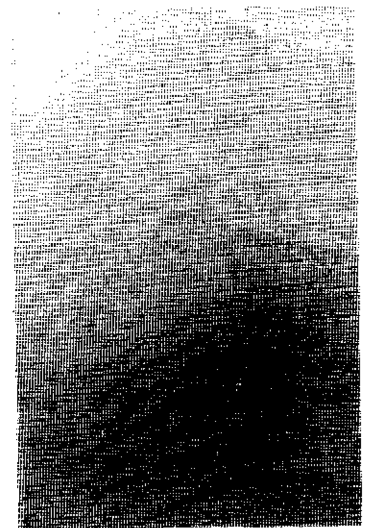

Fig. 2: Migration of the spleenic T-cells without antig

Results of the challenge experiments revealed tha inactivated sonicated vaccine gave 100 per cent prote to the challenge chicks. Their faeces were normal a clinical sign was recorded. The oocyst appeared in faeces on day 7 post challenge showing 200 oocy gram of faeces, which gradually increased to 900 o 13 post infection. In the non-vaccinated group, the o count per gram ranged from 25,000 to 30,000 at and 80,000 to 95,000 by the end of experiment oocyst count per gram of faeces was significantly hi ($P<0.01$) in control group as compared with the vacc

groups. It is evident from the results that the sonicated inactivated vaccine induced the cell mediated immune response as the vaccinated chicks resisted the heavy dose of challenge and gave 100 per cent protection.

Table 1: Results of splenic cell migration inhibition with and without antigen.

Chickens	Distance (micrometer)		Migration Index
	With antigen	Without antigen	
Immunized			
1	345.0	632.5	54.54
2	402.5	690.0	58.33
3	287.5	747.5	38.46
4	315.5	722.0	43.69
5	335.0	702.0	47.72
Unimmunized			
(Control) 1	775.5	1025.5	75.64
2	810.0	1278.0	63.38
3	845.5	1301.5	64.96
4	755.5	998.0	75.70
5	796.0	1015.5	78.38

It is concluded that modified splenic cell migration inhibition test can successfully be used to detect cell mediated immunity in chicken vaccinated against coccidiosis.

References

Akhtar, M., S. Ayaz, C.S. Hayat and M. Ashfaque, 1998. Immune response of sonicated coccidial oocyst in chickens. Pak. J. Biol. Sci., 1: 389-391

Barrett, J.T., 1988. Text Book of immunology and introduction to immunochemistry and immuno-biology. 5th ed. The C.V. Mosby Company, Washington, D.C.

Carpenter, R.R., 1963. *In vitro* studies of cellular hypersensitivity specific inhibition of migration of cells from adjuvant-immunized animals by purified protein derivative and other protein antigens. J. Immunol., 91: 803-818.

Gorden, H. Mec and H.V. Whitlock, 1939. A new technique for counting nematode eggs in sheep faeces. J. Coun. Sci. Industr. Res. Aust., 12: 50.

Jones, D.L. and G.M. Buening, 1983. *Brucella abortus* antigen induced lymphocyte reactivity in guinea pigs. Vet. Immunol. Immunopathol., 4: 433-443.

Klesus, P., W. Johnson and T. Kramer. 1977. Delayed wattle reaction as a measure of cell mediated immunity in chicken. Poul. Sci., 56: 249-256.

Morita, C. and N. Soekawa, 1972. Specific inhibition of splenic cells migration in chickens sensitized to delayed hypersensitivity. Poul. Sci., 50: 1503-1505.

Morita, C., Y. Tsutsmi and M. Soekawa, 1973. Migration inhibition test of splenic cells of chickens infected with *Eimeria tenella*. J. Parasitol., 59: 199-200.

Roitt, I., J. Brostoff and D. Male, 1998. Immunology. 4th ed. Mosby, USA.

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