OnLine Journal of Biological Sciences 2(8): 554-555, 2002
ISSN 1608-4127

ELISPOT Assay for Detection of IgA Secreting Splenic Cells in Chicken

Masood Akhtar, M. Mazhar Ayaz, C.S. Hayat, Abdul Jabbar, M. A. Hafeez, [1]M. Ashfaque and [1]I. Hussain
Immunoparasitology Laboratory, Department of Veterinary Parasitology
[1]Department of Veterinary Microbiology, University of Agriculture, Faisalabad, Pakistan

Abstract: The involvement of secretary immunoglobulin system in the protection of mucous surfaces by locally produced antibodies lead to the demonstration of IgA. IgA in the intestine of immune birds may limit coccidian replication. Enzyme linked immunospot (ELISPOT) assay was employed to detect the mucosal immune response (IgA antibody secreting splenic cells) in chicken vaccinated with egg adapted gametocytes antigen. Twenty to twenty-five % (200,000 to 250,000/10^6 cells) of chicken splenic cells stimulated with gametocytes antigen were detected as IgA secretors. Results of the ELISPOT revealed that spleen is one of the major source of cells producing IgA in chickens. Such a high number of IgA antibody secreting cells indicate protection in birds vaccinated with gametocytes antigen via oral route against coccidiosis. ELISPOT assay is found to be an advantageous alternative to conventional haemolytic plaque technique, being simpler and of equivalent or even greater sensitivity.

Key words: ELISPOT, splenic cells, coccidiosis, chicken, gametocytes

Introduction

Haemolytic plaque-forming cell (HPFC) assay have been described to detect single antibody-secreting cells (Jerne and Nordin, 1963). However these procedures have not found general acceptance, probably because of the technical difficulties that are encountered in trying to adapt HPFC assays to detect cells producing antigenic metabolites (Truffa-Bachi and Bordenave, 1980; Burns and Pyke, 1981). In addition, HPFC assays rely on the availability of developing antibodies that must bind complement which has seriously narrowed the applicability of this methodology. A method based on immunoenzyme technology was developed for enumerating antibody-secreting cells (Sedgwick and Holt, 1983; Czerkinsky *et al.*, 1983); termed as enzyme linked immunospot (ELISPOT) assay. The original ELISPOT assay was modified by using nitrocellulose membranes as solid support (Moller and Borrebaeck, 1985) instead of polystyrene. This paper reports the application of ELISPOT assay for the detection of cells, secreting IgA specific antibody, in spleen obtained from chicken immunized with coccidial gametocytes antigen.

Materials and Methods

Splenic cells: Spleens were removed from 10 chickens immunized with egg adapted *Eimeria tenella* gametocyte antigen on 5th day post vaccination. Each spleen was crushed over by pressing on the fine mesh in petriplates containing RPMI-1640. The suspension was then passed through the Nylon cell strainer (70 μm; Becton, Dickinson, Lincoln Park, NJ). Filtrate was centrifuged (1200 rpm/10min/4°C) and sediment was collected. Lysis buffer (1mL/spleen) was added for erythrocyte lysis and placed on ice for 2 min. Suspension was again passed through the cell strainer; centrifuge and collect the sediment. Took 10 μl of cell suspension and added equal amount of trypan blue. Counted the number of cells in the haemocytometer and adjusted its concentration with RPMI-1640 at 10^6 cells/100 μl in RPMI-1640.

Test procedure: Ninty-six well nitrocellulose-baked microtiter plates (Millipore Multiscreen MAHA N4550) were used to perform ELISPOT following the method of Czerkinsky *et al.* (1991).

- **Coating with Ig-specific primary antibody**: Plates were coated with Goat anti-chick Ig(H+L) UNLB (Primary antibody). Individual wells of the plate were filled with 100 μl of containing primary antibody at a final concentration of 2μg/mL. Plates were allowed to stand overnight at 4°C in humid chamber. Unadsorbed antibodies were removed by three successive washings with PBS. Wells were immediately filled with 100 μl RPMI-1640 to saturate remaining binding sites and incubated at 37°C for 2 hours. Medium was discarded and plate was dried by absorbant paper.

- **Incubation of Ig-secreting cells**: A 100 μl cell suspension containing 10^6 cells was dispended into each well in duplicate. Plates were then incubated undisturbed at 37°C for 4 h. The plates were rinsed twice with PBS by manual shaking and then thrice by immersion in PBS containing 0.05 % Tween 20 (PBST) for 2-3 min. The plates were emptied from wash buffer and the outer surface of the plates were carefully dried.

- **Addition of labeled secondary Ig-specific antibody**: A 100 μl of PBST containing Goat Anti-chick IgA-AP (1000-fold dilution) was added to each well and plates were incubated at 4°C overnight. The plates were then rinsed thrice by immersion in PBST and dried. Each well was then filled with 100 μl BCIP/NBT solution prepared by adding 50 μl of NBT (containing 50μg/mL nitroblue tetrazolium in 70 % N,N-Dimethylformamide) and 50 μl of BCIP (50μg/mL 5-bromo-4-choloro-3-indoyl phosphate in 100 % N,N-Dimethylformamide) in Alkaline phosphate buffer (containing 5.8 gm NaCl, 01 gm $MgCl_2$, 12.1gm Tris).

Plates were incubated for 10-25 min at room temperature (26°C) until blue spots developed. Plate were thoroughly washed with running tap water and air dry for 24h. The spots were counted under the dissecting microscope. Spots showing fuzzy boarders were considered positive.

Results and Discussion

Since the original description of the ELISPOT assay (Sedgwick and Holt, 1983; Czerkinsky *et al.*, 1983), a number of reports have

Corresponding Author: Masood Akhtar, Immunoparasitology Laboratory, Department of Veterinary Parasitology, University of Agriculture, Faisalabad, Pakistan

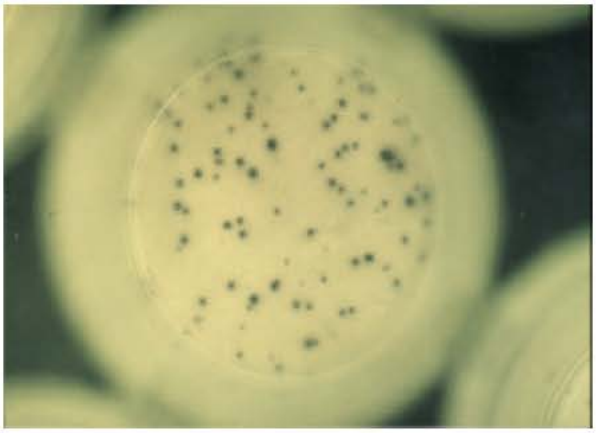

Fig. 1: Ig A antibody secreting cells detected by ELISPOT assay

attested to the versatility of this methodological principle for enumerating cells secreting antibodies, cells (lymphoid and non-lymphoid) secreting immunoreactive substances (Sedgwick and Holt, 1988; Czerkinsky *et al.*, 1988) and cells producing lymphokines and monokines (Czerkinsky, *et al.*, 1991). In the present study, ELISPOT assay was employed to detect the mucosal immune response (IgA antibody secreting cells) in chicken vaccinated with egg adapted gametocytes antigen (Akhtar *et al.*, 2002). Twenty to twenty-five % (200,000 to 250,000/10^6 cells) of chicken splenic cells stimulated with gametocytes antigen were detected as IgA secretors (Fig. 1). Results of the ELISPOT revealed that spleen is one of the major source of cells producing IgA in chickens as reported by Czerkinsky *et al*. (1983). Similar studies in chickens on gut-associated lymphoreticular tissue (GALT) including bursa of fabricius, cecal tonsils, peyer's patches and lamina propria are needed to investigate the source of IgA secreting cells. The involvement of secretary immunoglobulin system in the protection of mucous surfaces by locally produced antibodies lead to the demonstration of IgA immunoglobulins in fowl secretions. It is documented in the literature that IgA containing preparations from the intestine of immune birds may limit coccidian replication (Rose, 1976). In the present study, such a high number of IgA antibody secreting cells is an indicative of protection in birds vaccinated with gametocytes antigen via oral route against coccidiosis.

ELISPOT assay was found to be an advantageous alternative to conventional haemolytic plaque technique, being simpler and of equivalent or even greater sensitivity.

References

Akhtar, M., M.M. Ayaz, C.S. Hayat, M. Ashfaque and I. Hussain, 2002. Adaptation of *Eimeria tenella* (local isolate) sporozoites in chicken embryos. Pak. Vet. J., In press.

Burns, G.F. and B.L. Pyke, 1981. Spontaneous reverse hemolytic plaque formation. I. Technical aspects of the protein assay. J. Immunol. Methods, 41: 269-275.

Czerkinsky, C., L.A. Nilsson, H. Nygren, O. Ouchterlony and A. Tarkowski, 1983. A solid phase enzyme linked immunospot assay (ELISPOT) for enumerating antibody-secreting cells. J. Immunol. Methods, 65: 109-113.

Czerkinsky, C., L.A. Nilsson, A. Tarkowski, W.J. Koopman, J. Mestecky and O. Ouchterlony, 1988. The solid phase enzyme-linked immunospot assay (ELISPOT) for enumerating antibody-secreting-cells: Methodology and applications. In: Theoretical and Technical Aspects of ELISA and other solid phase immunoassays, eds. Kemeny, D.M. and Challacombe, S.J., John Wiley, Oxford.

Czerkinsky, C., G. Andersson, B. Ferrua, I. Nordstrom, M. Quiding, K. Eriksson, L. Larsson, K. Hellstrand and H. Ekre, 1991. Detection of human cytokine-secreting cells in distinct anatomical compartments. Immunol. Rev., 119: 1-22.