

Abrogation of attachment of Infectious Bursal Disease Virus to chicken B cells by treatment of B cells with Soyabean Agglutinin and Wheat Germ Lectin

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Abstract

Infectious Bursal Disease (IBD) is a viral disease of young chickens with high morbidity and low mortality. The target molecule on B cells for binding of IBD virus (IBDV) is not known. In this study, bursal B cells from chicken aged 6 weeks were treated with either Soyabean Agglutinin (SBA) or Wheat Germ Lectin (WGL) prior to incubation with IBDV. The mean percentages of B cells positive for IBDV binding without and after treatment of B cells with lectins SBA and WGL were 94.48 ± 0.47 , 88.52 ± 0.54 and 88.86 ± 0.35 , respectively. The differences between the values without and after treatment in case of both the lectins were found to be highly significant ($p < 0.0001$). However, the two lectins did not differ significantly with each other in their effects. ANOVA among the values of the three groups revealed very significant differences ($F = 52.778$; $p = 0.0005$) among them. The results suggest that the putative IBDV binding site on B cells may possibly be a lectin - binding molecule.

INTRODUCTION

Infectious Bursal Disease (IBD) is a major immunosuppressive disease of chickens having serious economic impact on the poultry industry worldwide. It is an acute, highly contagious disease of young chickens of 3 – 6 weeks of age caused by the Infectious Bursal Disease Virus (IBDV) belonging to the genus *Avibirnavirus* of family *Birnaviridae* (Dobos et al., 1979; Murphy et al., 1999). IBDV infection causes depletion of B cells by apoptosis (Jungmann et al., 2001). Although sIgM – bearing pre-B cells have been shown to be vulnerable for IBDV infection (Hirai and Calnek, 1979), the exact identity of the target receptor for IBDV attachment on pre-B cells is not known. If the specific molecule which serves as the target for IBDV attachment to B cells, could be identified, it will pave the way for effective control of IBD by blocking the interaction of IBDV with its ligand on B cell surface through immunological means or recombinant target protein. The present study was, therefore, undertaken to investigate the possible affinity of IBDV towards certain lectin – binding molecules on bursal B cells.

MATERIALS AND METHODS

The guidelines of the Institutional Animal Ethics Committee were followed in all the experiments.

Experimental birds: Day – old White Leghorn male chicks were procured from the Hatchery of the Department of Animal Breeding and Genetics, PAU, Ludhiana and reared in the Animal House of the Department of Veterinary Microbiology till used at the age of 6 weeks.

Virus preparation: The intermediate D-78 vaccine strain of IBDV was propagated in BGM-70 cells.

Cell culture for propagation of virus: The BGM-70 cell line was gifted by Dr. Y. M. Saif and Dr. Robert Dearth, Ohio State University, Ohio, USA. The cells were cultured in Eagle's Minimum Essential Medium. The cells were grown to confluence in tissue culture flasks. The growth medium was removed and the cell monolayer was washed twice with the maintenance medium. Virus stock in maintenance medium (0.2 ml) was inoculated in the culture at 37°C and monitored daily for cytopathic effects upto 5 days. At 5 days post-inoculation, the monolayer was disrupted by repeated freeze – thaw cycles and the suspension was clarified by low speed centrifugation. The supernatant fluid was harvested and the cell culture lysate was used as virus inoculum in the subsequent passage. The monolayer was infected and the second passaged virus / cell lysate was harvested, aliquoted and stored at –200°C until used further.

Collection of bursae from chicks: Healthy young chicks aged

6 weeks were slaughtered by cervical dislocation. Bursae were then immediately excised from the dead birds and collected in Hank's Balanced Salt Solution (HBSS).

Preparation of bursal cells: Immediately after collection, each bursa was thoroughly rinsed in HBSS, dissected free of fat and capsule and transferred to fresh HBSS. After removing capsule it was finely chopped with scissors. Minced tissue was gently forced through 60 µm pore sized steel wire mesh to obtain a single cell suspension. It was also passed through 24G needle to dissociate cellular aggregates into individual cells.

The lymphoid and non-lymphoid bursal cell populations were separated by density gradient centrifugation using Ficoll Hypaque (Sigma, USA). After centrifugation, the white lymphocytic cell layer at the junction of the two fluids was harvested carefully and the bottom pellet of non-lymphoid bursal cells was discarded. The bursal mononuclear cells were incubated at 37°C for 1 hour on a plastic surface to avoid macrophage contamination. After incubation, the supernatant containing the lymphocytes was collected and used further. The concentration of cells with more than 90% viability was adjusted to 2×10^6 cells/ml.

Lectin treatment of B cells: B cell binding lectins – Soyabean Agglutinin (SBA) and Wheat Germ Lectin (WGL) (Genei, Bangalore) were used to study the inhibition of virus binding to bursal B cells. The working concentration of SBA used in the present study was 1 µg/ml in Phosphate Buffered Saline (PBS) (Reisner et al., 1980). In case of WGL, the working concentration used in the present study was 10 µg/ml in PBS (Damm et al., 2004; Walter et al., 2001). The bursal B cell suspension (2×10^6 cells/ml; 0.5 ml) was centrifuged at 1000 rpm for 10 min. The supernatant was discarded and the cell pellet was resuspended in sufficient quantity of 3% H_2O_2 in methanol for 20 min. to inactivate the endogenous peroxidase. After washing, the cell pellet was incubated with 50 µl of working solution of either of the two lectins i.e. SBA or WGL for 1 hr. at 37°C. Control cells were incubated with 50 µl of PBS only.

The IBDV preparation was diluted (1:10) in PBS and 100 µl of the virus suspension was allowed to adsorb over B-lymphocytes for 1 hour at 37°C. Two washings were then given with 0.05 M PBS (pH 7.4). The cell pellet was then resuspended in the blocking solution i.e. 1% Bovine Serum Albumin (BSA) in PBS and incubated at 37°C for half an hour to check the nonspecific background staining.

Immunochemical staining: Immunoperoxidase staining was employed for the detection of IBDV binding to B-lymphocytes. The standard Immunoperoxidase staining protocol (Cho et al., 1987) was followed with certain minor modifications.

The cell suspension was centrifuged at 1000 rpm for 10 min and washed to remove the Blocking Solution. The cells were then incubated with 100 µl of rabbit anti-IBDV antibodies diluted (1:10) in PBS at 37°C for one and a half hours followed by two washings with PBS. The cells were then suspended in 50 µl Biotinylated Goat anti-rabbit antibody (Genei, Bangalore) diluted (1:40) in PBS and incubated at 37°C for one hour followed by two washings. The cells were then suspended in 50 µl of Avidin-HRPO (Genei, Bangalore, diluted (1:40)) in PBS and incubated at 37°C for half an hour followed by two washings with PBS. The cells were then treated with the solution containing 5 mg Diaminobenzidine tetrahydrochloride (DAB) in 10 ml of Tris buffer (0.05 M, pH 7.5, 0.01% H_2O_2) for 10 min. After development of brown color, the cells were washed twice with distilled water. The cells were finally suspended in 100 µl of PBS.

The number of stained as well as unstained B cells were counted under a light microscope in both test as well as control, to determine the extent of inhibition of virus binding after treatment of B cells with the lectins.

RESULTS AND DISCUSSION

The role of lectin-binding molecule(s) on B cell surface as possible target(s) for IBDV binding was studied by treating B cells with either of the two lectins, Soyabean Agglutinin (SBA) or Wheat Germ Lectin (WGL) prior to incubating with IBDV. The mean (+ SE) values of percentages of B cells positive for IBDV binding without and after treatment of B cells with lectins SBA and WGL were 94.48 ± 0.47 , 88.52 ± 0.54 and 88.86 ± 0.35 , respectively (Table 1). The differences between the mean values without and after treatment in case of both the lectins were found to be extremely significant ($p < 0.0001$). However, the difference between the two lectins in the values after treatment was not significant. ANOVA among the three groups (i.e. untreated, SBA-treated and WGL-treated) revealed very significant differences ($F = 52.778$; $p = 0.0005$) among them.

Figure 1

Table 1: Effect of treatment of B cells with lectins on IBDV binding to B cells

S. No.	Percentage of B cells positive for IBDV binding		
	Untreated B cells	SBA treated B cells	WGL treated B cells
1	90	84	87
2	91	86	88
3	92	85	87
4	92	88	89
5	93	90	88
6	93	87	86
7	93	90	87
8	93	88	90
9	94	90	90
10	95	87	89
11	95	90	86
12	95	87	90
13	95	84	90
14	96	92	91
15	96	92	89
16	96	87	90
17	97	91	89
18	97	91	91
19	97	89	90
20	97	91	88
21	97	90	91
Mean \pm SE	94.48 \pm 0.47	88.52 \pm 0.54	88.86 \pm 0.35

The studies with the two B cell - specific lectins have yielded interesting results, which suggest that the putative IBDV-binding target on B cell may possibly be a lectin-binding glycoprotein or a carbohydrate derivative. Treatment of B cells with either of the two types of lectins resulted in an extremely significant decrease in IBDV binding to B cells. However, the two lectins did not differ significantly with each other in their effect on B cells suggesting that the activity may possibly be due to a functional site similar in both the lectins binding the same molecule on the B cells.

Cellular interactions within the immune system are in part mediated via the carbohydrate-rich coat of the cell membrane, the glycocalyx, of which the terminal carbohydrate residues are of particular functional importance. These carbohydrate residues from bursa of 2- and 30- day old chickens were investigated by lectin histochemistry in an earlier study. The brush border of the lining epithelium, the macrophages and the endothelium were labelled by mannose-specific lectins. The follicle – associated epithelium was labelled by a broad spectrum of lectins. Epithelial cells that separated the cortex from the medulla and large mononuclear cells in the cortex were only

labeled by N-acetyl galactosamine-specific and N-acetyl glucosamine-specific lectins, respectively (Gastman et al., 2004).

In a study (Ogawa et al., 1998), IBDV bound to 94% cells in the lymphocytes prepared from bursa Fabrici. Most of the cells, which bound the virus, were sIgM positive, but a small number of them were sIgM negative. The binding of IBDV to the chicken B lymphoblastoid cell line LSCC-BK3 cells was affected by treatment of the cells with proteases and N-glycosylation inhibitors. It was suggested that the IBDV host range is mainly controlled by a virus receptor composed of N-glycosylated protein associated with the subtle differentiation stage of B-lymphocytes represented mostly by sIgM – bearing cells. The chB1 gene expression indicates active bursal B-lymphocytes. The surface expression of a carbohydrate structure Lewis-X identifies those bursal B-lymphocytes that are undergoing gene hyperconversion (Ivan et al., 2001).

Programmed cell death or apoptosis is a common mechanism for selective elimination of cells during development of many animal cell lineages. Developing B cell population in the avian bursa of Fabricius is highly susceptible to programmed cell death. It was suggested (Neiman et al., 1991) that the bursal microenvironment is required for the rapid expansion of the developing B cell mass. In avians, cell contacts or short-range association within the architecture of bursal follicles served to restrain and modulate the tremendous proliferative capacity of bursal lymphocytes. They speculated that the induced apoptosis was a vital mechanism to avoid potentially disastrous consequences of unrestrained growth of B cells. It was demonstrated (Compton and Waldrup, 1998) that the endogenous activation of bursal lymphocyte apoptosis might be mediated by protein kinase C signal transduction pathway.

The lymphocyte populations of bursal follicles, which are normally subjected to high rates of physiological death, were found (Neiman, 2005) to be sensitive to loss of cell-cell contact. It was also found that Nr-13, a member of Bcl-2 family, was expressed in embryonic bursal cells but it was shut off after hatch, when the bursal stem cell population disappears.

Even though all lymphocytes have lectin receptors, B lymphocytes were found (Dios et al., 1983) to be more specific for Lens culinaris lectin (LC), Pisum sativum lectin

(PS), Glicin max lectin (SBA), Dolichos biflours lectin (DB), Ricinus communis lectin (RCA II) and Triticum vulgaris lectin (WGA), respectively.

The relationship between the degree of lectin-cell binding, cytotoxicity and cytoagglutinating activity of plant derived lectins in normal lymphocytes has been studied (Ohba and Bakalova, 2003). The WGA at concentrations higher than 0.05 M showed high toxicity against normal lymphocytes.

It has been found (Gastman et al, 2004) that WGA initiated an accelerated type of programmed cell death development after only 30 min of incubation with tumor cells and suggested that WGA could induce apoptosis by binding to either N-acetylneuraminic acid or N-acetyl glucosamine on the cell surface. WGA induced a loss of transmembrane potential, disruption of the inner mitochondrial membrane and release of cytochrome C and caspase-3 activation after 30 minutes of cell interaction. Thus, WGA triggers a unique pattern of apoptosis that is extremely fast, Fas- and Caspase-3- independent, and is mediated via the mitochondrial pathway.

It is speculated that the lectin binding moiety of B cells which has been shown in our study to be the possible IBDV binding site, may be the key for induction of apoptosis in bursal B cells by IBDV. The lectin – binding moiety on the B cells which appears from the results of our study to be a possible target for IBDV binding, needs to be characterized further. This should help in devising appropriate strategies for better prophylaxis and control of IBD.

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