Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

Distribution of Lymphocytes in the Mucosa Associated Lymphoid Tissues (MALT) of Naturally Occurring Infectious Bursal Disease (IBD) in Chicken

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ARTICLE HISTORY

Received: August 18, 2009
Revised: November 02, 2009
Accepted: January 01, 2010

Key words:
Chicken
Distribution
IBDV
Lymphocytes
MALT

ABSTRACT

This study was aimed to investigate changes in the number and distribution of lymphocytes in the mucosa associated lymphoid tissues (MALT) of digestive tract (proventriculus, duodenum, jejunum, ileum, cecum and cecal tonsils) and respiratory system (lungs) of chicken infected by Infectious Bursal Disease Virus (IBDV). Samples were divided into two groups; IBDV infected group (21, 24 and 30 days old) and control group (non infected birds; 21 days old). Haematoxylin and eosin stained slides were prepared for microscopic studies to observe the distribution and the number of lymphocytes in the mucosa of the digestive tract and respiratory system. Lymphocytes were significantly ($P<0.05$) lower in proventriculus, duodenum, jejunum, ileum, cecum, cecal tonsils and lungs of IBDV infected chickens than the control. Moreover, the reduction in lymphocytes number was maximum in duodenum and cecal tonsils, while minimal in lungs. Depletion of lymphocyte was mainly in the lamina propria and the core of the villi and depletion increased with the advance of age of IBDV infected chicken. These results demonstrate that IBDV destroys the lymphocytes of the MALT and suppresses the immunity.

To cite this article: Uddin MM, MZI Khan, KN Islam, ASMG Kibria, GN Adhikary, MNH Parvez, J Basu, MB Uddin and MM Rahman, 2010. Distribution of lymphocytes in the mucosa associated lymphoid tissues (MALT) of naturally occurring infectious bursal disease (IBD) in chicken. Pak Vet J, 30(2): 67-71.

INTRODUCTION

In chicken, the digestive system, respiratory system, urinary system and the reproductive system are mainly lined by mucosa, which forms a barrier between the external and internal environments. When mucosa is exposed to foreign antigens, the mucosa associated lymphoid tissues (MALT) act as a source of lymphocytes, polymorphonuclear leukocytes, plasma cells and macrophages. This tissue plays an important role in immunological response to viruses as well as helps to induce immunity after oral immunization (Arai *et al.*, 1988; Stitz, 1994). Immune competent cells including lymphocytes, plasma cells and macrophages have the ability to develop an immune response following exposure to antigens (Anderson, 1989). Lymphocytes are distributed homogenously in lymphatic nodules in the mucosa associated lymphoid tissues.

Infectious bursal disease has been of great economic importance for the developing poultry industry (Alkhalaf, 2009). The infectious bursal disease virus (IBDV) at first infects (replicates) immune competent cells (lymphocytes and macrophages) in the mucosa associated lymphoid tissues of the duodenum, jejunum and cecum and subsequently replicates in the immature B-lymphocytes of Bursa of Fabricius and causes immunosuppression in chicken (Breytenbach, 2003). This immunosuppression prevents birds from optimally responding to vaccines (Winterfield and Thacker, 1978).

The distribution of immune competent cells of the mucosa-associated lymphoid tissues (MALT) and other major lymphoid organs of the chicken have previously

been reported (Vervelde and Jeurissen, 1993; Khan and Hashimoto, 1996; Khan *et al*., 1998; Khan and Hashimoto, 2001). Moreover, the histopathological changes and immunosuppressive effects on different lymphatic tissues of IBDV infected chicken have also been reported in Bursa of Fabricius (Tsukamoto, *et al*., 1995; Elankumaran *et al*., 2001; Alkhalaf, 2009), spleen (Hoque *et al*., 2001), thymus (Hoque *et al*., 2001), cecal tonsils (Elankumaran *et al*., 2001) and other non lymphoid organs like kidneys (Van der Sluis, 1994) and liver (Islam *et al*., 1997; Chowdhury *et al*., 1996).

However, relatively little information is available regarding the changes in number and distribution of lymphocytes in the mucosa-associated lymphoid tissues (MALT) of IBDV infected chicken. The present paper describes the distribution of lymphocytes in MALT of IBDV infected chickens. These investigations will provide valuable information to poultry immunologists, pathologists, researchers and anatomists.

MATERIALS AND METHODS

Samples from 30 IBDV infected chicken were collected immediately after postmortem examination of diseased chicken on the basis of gross lesions at the Department of Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh. These IBDV infected chickens of different ages were received from different commercial poultry farms for the diagnosis of the disease. Samples were divided into two groups; IBDV infected group and control group (non infected birds; 21 day old) as reference value. Birds of IBDV infected group were further divided into 3 groups according to their age viz. 21, 24, and 30 days and each group had 10 birds. Sample of all the groups (IBDV infected and control) were collected and processed for the microscopic studies. Different segments of digestive tract (proventriculus, duodenum, jejunum, ileum, cecum, and cecal tonsils) and lung tissue were fixed in Bouin's fluid. Tissue samples were dehydrated in alcohol, cleaned in xylene, embedded in paraffin, sectioned at 5µ thickness, stained with Harris's Haematoxylin and Eosin and mounted with DPX (Gridley, 1960). Changes in number and distribution of lymphocytes in the MALT of the digestive tract and respiratory tract of IBDV infected chicken were studied and counted in 20 microscopic fields selected randomly using high magnification (X 400 and 1000). The data were statistically analyzed using analysis of variance technique by using SPSS 12 statistical software. The suitable photographs from the selected specimens were prepared and placed for better comparison and illustration of the results.

RESULTS AND DISCUSSION

Proventriculus

The numbers of lymphocytes in non infected 21 day old chicken were 35.70 ± 1.59, while in IBDV infected 21, 24 and 30 days old chicken these values were 27.70 ± 1.09, 23.60 ± 1.17 and 27.60 ± 1.46, respectively (Table 1). Lymphocytes were significantly ($P<0.05$) lower in IBDV infected than the control group at day 24 of age. Lymphocytes were reduced and depopulated specially in the lamina propria of IBDV infected chicken of all age groups. A report in this regard shows that after oral infection or inhalation, the virus replicates primarily in the gut-associated lymphocytes and macrophages (Muller *et al.*, 1979; Befus *et al*., 1980). It can be speculated that IBDV may replicate and destroy the lymphocytes of proventriculus.

Duodenum

In the duodenum, numbers of lymphocytes were 44.90 ± 1.83 in 21 days old non-infected chicken, while 34.70 ± 1.93, 32.50 ± 1.15 and 31.60 ± 1.63 in 21, 24 and 30 days old IBDV infected chicken, respectively (Table 1). The values were significantly ($P<0.05$) lower in IBDV infected than non-infected control chicken at 24 and 30 days of age. Severe depletion of lymphocytes was observed in the lamina propria and the core of villi of IBDV infected chicken than the non-infected controls (Fig. 1a & b). These results are indirectly similar to the earlier findings that the virus replicates in the lymphocytes of duodenum as the first site demonstrated by using immunofluo-rescence techniques (Weiss and Weiss, 1994).

Jejunum

Lymphocytes were significantly ($P<0.05$) lower in number in the jejunum of IBDV infected chicken of age groups of 24 and 30 days than the non infected control (Table 1). Depletion of lymphocytes was observed in the core of villi and in the lamina propria of jejunum of the infected chicken compared to non-infected control (Fig. 3a & b), which might be due to the mechanism of IBDV infection. The virus replicates primarily in the lymphocytes and macrophages of gut associated tissue in the jejunum after oral infection or inhalation (Muller *et al.*, 1979; Weiss and Weiss, 1994).

Ileum

Lymphocytes were 26.20 ± 1.56, 26.50 ± 1.27 and 26.50 ± 1.37 in 21, 24 and 30 days old IBDV infected chicken respectively, while 31.80 ± 1.48 in 21 days old non infected chicken (Table 1). Lymphocytes were lower in infected groups than the control group, the difference was, however, non significant. Disorganization and depletion of lymphocytes were similar in all age groups of IBDV infected chicken. Moreover, severe disorganization and depletion of lymphocytes were most common in the lamina propria of ileum. IBDV proliferates in immature B cells within the follicles of specialized gut-associated lymphoid organs (Befus *et al.*, 1980; Withers *et al.*, 2006).

Cecum

The lymphocytes were significantly ($P<0.05$) higher in the non-infected chicken than the IBDV infected chicken of 24 and 30 days of age (Table 1). Nevertheless, lymphocytes were depleted at 30 days in IBDV infected chicken than 24 day and 21 day old IBDV infected chicken, especially in the lamina propria of cecum. These findings are in agreement with the mechanism of IBDV infection, as the virus replicates in the lymphocytes in the lamina propria of cecum (Muller *et al.*, 1979; Weiss and Weiss 1994).

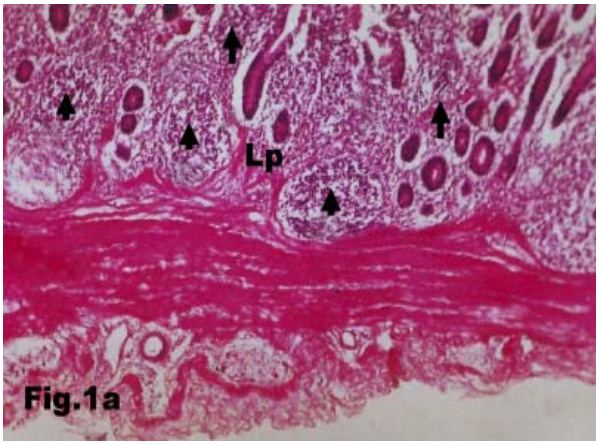

Fig. 1a: Duodenum of 21 days old non-infected chicken showing lymphatic nodules (arrow heads) and diffuse lymphocytes (arrows) in the lamina propria (Lp, H & E, X 400).

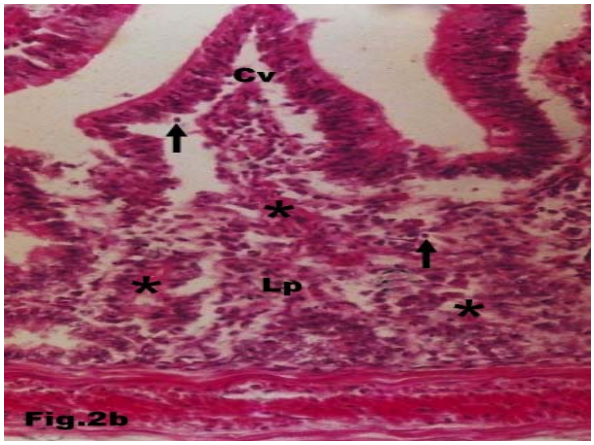

Fig. 2b: Jejunum of 21 day old IBDV-infected chicken showing lymphocytes (arrows) and lymphocyte depleted areas (asterisks) in the lamina propria (Lp) and core of villi (Cv, H & E, X 1000).

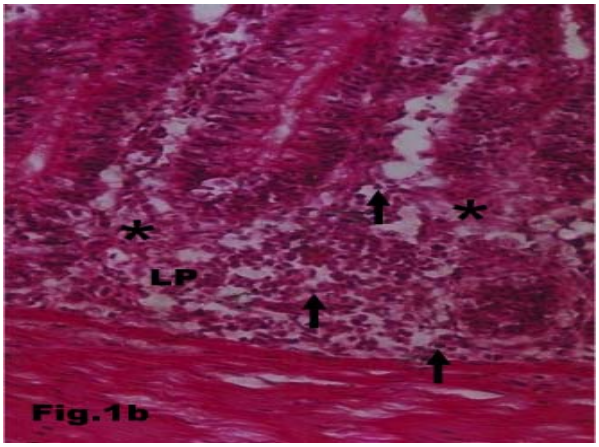

Fig. 1b: Duodenum of 21 days old IBDV infected chicken showing lymphocytes (arrows) and lymphocyte depleted areas (asterisks) in the lamina propria (Lp, H & E, X 1000).

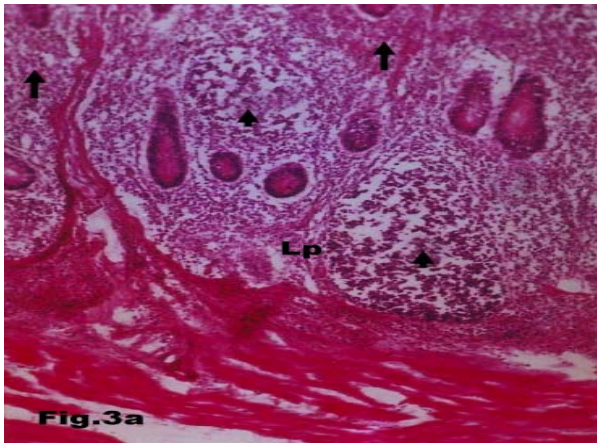

Fig. 3a: Cecal tonsil of 21 day old non-infected chicken showing diffuse lymphocytes (arrows) and lymphatic nodules (arrow heads) in the lamina propria (Lp, H & E, X 400).

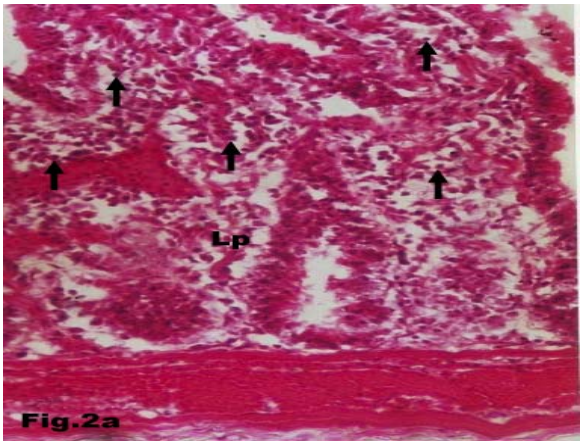

Fig. 2a: Jejunum of 21 day old non-infected chicken showing lymphocytes (arrows) in the lamina propria (Lp, H & E, X 1000).

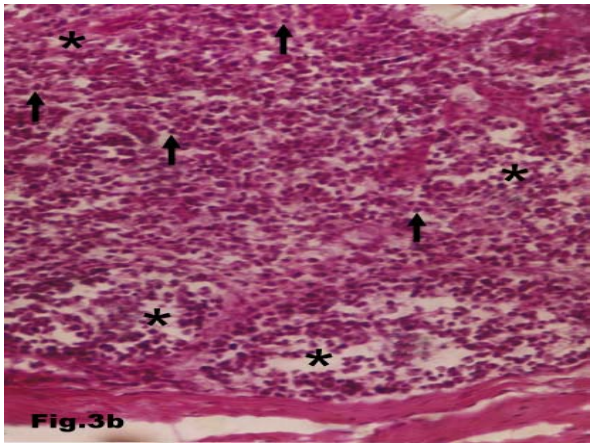

Fig. 3b: Cecal tonsil of 21 day old IBVD-infected chicken showing lymphocytes (arrows) and lymphocyte depleted areas (asterisks), H & E, X 1000.

Table 1: Distribution of lymphocytes in mucosa associated lymphoid tissue of chicken (mean ± SD, n = 10).

Group	Organs						
	Proventriculus	Duodenum	Jejunum	Ileum	Cecum	Cecal tonsil	Lungs
Control							
Day-21	35.70 ± 1.59	44.90 ± 1.83	37.60 ± 2.34	31.80 ± 1.48	36.60 ± 1.27	63.70 ± 1.57	37.90 ± 0.76
IBDV Infected							
Day-21	27.70 ± 1.09	34.70 ± 1.93	31.80 ± 1.65	26.20 ± 1.56	31.20 ± 1.31	53.00 ± 1.21	35.30 ± 1.83
Day-24	23.60 ± 1.17*	32.50 ± 1.15*	29.40 ± 1.08*	26.50 ± 1.27	28.00 ± 1.05*	48.50 ± 1.42*	30.80 ± 1.75*
Day-30	27.60 ± 1.46	31.60 ± 1.63*	28.50 ± 1.35*	26.50 ± 1.37	23.70 ± 1.95*	42.40 ± 2.72*	28.30 ± 2.04*

* Significantly different from control (P<0.05).

Cecal tonsil

Lymphocytes in non-infected chicken of 21 days of age were 63.70 ± 1.57 and in infected groups these values were 53.00 ± 1.21, 48.50 ± 1.42 and 42.40 ± 2.72 on 21, 24 and 30 days of age, respectively (Table 1). Lymphocytes were significantly (P<0.05) reduced in number in the cecal tonsils of IBDV infected chicken at 24 and 30 days of age compared to controls. Disorganization of lymphocytes in the tonsiler nodules and severe depletion in the diffuse form were observed in the IBDV infected chicken of all age groups (Fig. 3a & b). The main target cells for IBDV replication seem to be the actively dividing B lymphocytes and the infection also leads to the destruction as well as death of lymphocytes in caecal tonsils (Nunoya *et al.,* 1992; Tanimura *et al.,* 1995; Chowdhury *et al.,* 1996; Chen *et al.,* 2009; Khatri and Sharma, 2009).

Lungs

Lymphocytes were also significantly (P<0.05) lower in number in the IBDV infected chicken of 24 and 30 days of age than the non-infected controls (Table 1). The plasma cells were reduced in the upper respiratory tract at 7 days post-inoculation, compromising local immunity (Dohms *et al*., 1988).

The result of the present study show that IBDV not only destroys the lymphocytes of the Bursa of Fabricius and other major lymphoid organs but also significantly (P<0.05) destroys the lymphocytes of the mucosa associated lymphoid tissues of digestive tract and to some extent of respiratory tract.

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