An Immunohistochemical Analysis of T-Cell Subsets in the Chicken Bursa of Fabricius during Postnatal Stages of Development

Mohammad Zahirul Islam KHAN and Yoshiharu HASHIMOTO

Laboratory of Anatomy, Department of Biomedical Science, Postgraduate School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan

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ABSTRACT. T-cell subsets in the chicken bursa of Fabricius were analysed immunohistochemically during postnatal stages. Distinct types of T cells were present both in the surface epithelium and lamina propria of the bursa. TcR1⁺ and CT8⁺ cells were predominant in the surface epithelium, while TcR2⁺ and CT8⁺ cells were numerous in the lamina propria. These T-cell subsets peaked in frequency of occurrence at 5 weeks. They then decreased in number, and a few T cells remained in the bursa until 15 weeks of age. This result showed that the bursa of chickens is furnished with the distinct types of T-cell subsets at early postnatal stages of development to maintain its local immunity. — **KEY WORDS:** bursa of Fabricius, chicken T cell, immunohistochemistry.

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The bursa of Fabricius, an organ unique to birds, arises in early embryonic life as a dorsal cloacal diverticulum. In the bursa B lymphocytes proliferate and are delivered throughout the body via peripheral blood circulation. These lymphocytes are engaged in synthesizing and storing different classes of immunoglobulins (Igs) [4, 12]. In addition to the production of B cells, the bursa is known to absorb antigens through the cloacal lips [8, 11]. Therefore, the bursa needs to be furnished with its own defense system. In this regard, only a few reports are available on distribution of T cells in this organ. Odend'hal and Breazile [10] noted the presence of a T cell-dependent area, designated the diffusely infiltrated area (DIA), located just dorsal to the bursal duct which connects the bursa with the cloaca. In the present study, we immunohistochemically surveyed T cells in the epithelium and lamina propria of the postnatal chicken bursa, because of its rapid development followed by subsequent atrophy, and seeding of post-bursal stem cells to other lymphatic tissues during these stages.

Chickens of the Dekalb strain were purchased from Central Chicken Breeding Centre, Uni-cho, Hokkaido and were sacrificed after proper anesthesia at 1 day, and at 5, 9, and 15 weeks of age (three chickens in each group). The bursal tissues were removed as indicated in Fig. 1, and snap frozen in liquid nitrogen for immunohistochemistry. For immunostaining the avidin-biotin-complex (ABC) method was used as described previously [6]. In brief, 4 μ m-thick frozen sections were placed on 0.5% Neopren (Nisshin EM, Japan)-coated glass slides, fixed in ice-cold acetone for 15 min, and kept at -20°C until use. The sections were incubated for 3 hr with monoclonal antibodies (mAbs) (CT3, CT4, CT8, TcR1, TcR2, or TcR3, all purchased from Southern Biotechnology Associates, U.S.A.), diluted with 0.01 M phosphate-buffered saline (PBS) at the appropriate working dilution (CT3, CT4, and CT8 mAbs were used at a dilution of 1:200, 1:100 and 1:100, respectively; TcR1, TcR2, and TcR3 mAbs were all diluted 1:500). After rinsing with PBS, they were overlaid with 1% biotin-conjugated goat anti-mouse Igs (Tago Immunologicals, U.S.A.) for 1 hr, followed by incubation with ABC solution (Vector Lab.,

U.S.A.) for 1 hr. The antigen-antibody reaction was visualized with 0.05 M Tris-HCl buffer (pH 7.6) containing 0.02% 3, 3' diaminobenzidine-tetrahydrochloride dihydrate (Kanto Chemical, Japan) and 0.03% H_2O_2 , and counterstained slightly with hematoxylin. Control sections were stained by the same procedure without the mAbs.

Cell count was carried out randomly in 20 fields in the lamina propria using an eyepiece micrometer of 19×19 squares (Olympus, Japan) at a magnification of $\times 400$. In the epithelium, T-cell subsets were counted in a total of 7220 smallest units of the same micrometer (equivalent to 20 fields) as described previously [6] at the same magnification, and their relative frequencies per 0.1 mm^2 area both in the epithelium and lamina propria were calculated according to point-counting technique [13].

Fig. 1. Diagrammatic representation of the bursa and hind gut of the chicken (a modification of Odend'hal and Breazile, 1989). The tissue samples used in the present study were obtained from region "S". B; bursa, C; cloaca, DIA; diffusely infiltrating area, and G; gut.

Counting of TcR3 was omitted because its frequency of occurrences were very low in the bursa. Relative frequencies between CT4 and CT8⁺ cells, and between TcR1 and TcR2⁺ cells in the epithelium and lamina propria were compared by the two-tailed Student's *t*-test [14].

Numerous lymphocytes present in the epithelium and lamina propria of the bursa were stained with mAb CT3, which is a pan T-cell marker. CT3⁺ cells were evenly located in all parts of the surface epithelium. In the lamina propria of the bursa, they were rich in the cortex of lymphoid

follicles. Dense distribution of CT3⁺ cells was also found in the interfollicular connective tissue and the subepithelial region (Fig. 2a). Among T-cell subsets, CT8⁺ cells were more numerous than CT4⁺ cells in both the surface epithelium and lamina propria (Figs. 2b-c, 3b, 4b). TcR1⁺ cells were more abundant than TcR2⁺ cells in the surface epithelium, while in the lamina propria TcR2⁺ cells were predominant. TcR3⁺ cells were rarely found in the bursal tissue (Figs. 2d-f, 3a, 4a). T-cell subsets were detected from one day (1d) to 15 weeks, being highest in frequency

Figs. 2a-f. Serial sections from the bursa of a 5-week-old chicken are immunostained with CT3 (a), CT4 (b), CT8 (c), TcR1 (d), TcR2 (e), and TcR3 (f) antibodies. In the lamina propria, CT8⁺ cells predominate over CT4, and TcR2⁺ cells are slightly more numerous than TcR1⁺ cells. TcR3-positive cells (Fig. 2f) (arrows) are very rare. C and M: cortex and medulla of a follicle, respectively; IFC: interfollicular connective tissue; PV: postcapillary venule; SE: subepithelial region; an asterisk means the surface epithelium. $\times 320$.

of occurrence at 5 weeks and thereafter decreasing in number (Figs. 3–4).

The present study is the first to demonstrate T-cell subsets in the chicken bursa under normal conditions. A previous immunohistochemical study failed to detect avian homologous molecules of mammalian CD4 (helper T cell) and CD8 (suppressor/cytotoxic T cell) in the bursa [7]. On the other hand, Bucy *et al.* [2] found T-cell subsets in the transplanted chicken bursa. According to them, when bursal fragments from the chicken were transplanted into the quail embryo, T cells expressing a CT3⁺ CT8⁺ TcR1⁺ TcR2⁺ TcR3⁺ phenotype were dispersed within the donor bursal fragments for a short period. The dense distribution of T cells in the bursal lamina propria, as shown in the present study, suggests that T cells supplied from the peripheral blood infiltrate into the bursa, possibly via post capillary venules at the cortico-medullary junction, and move further into the epithelial region. This idea is not in agreement with Odend'hal and Breazile [9], who reported that T cells existing in the bursa are supplied from the DIA only. T-cell subsets in the bursa appeared at the age of 1 day, peaked in frequency at 5 weeks, and then gradually decreased in number. Glick [3] observed the maximum growth of the chicken bursa at around 4 weeks of age (in comparison to the body weight), and from then the rate of growth begins to fall slowly until 10 weeks. Therefore, it is supposed that the traffic of T cells into the bursa is influenced by the age-

dependent growth and subsequent atrophy of the bursa.

In the present study, TcR1⁺ cells and CT8⁺ cells were more abundant in the epithelium. Janeway *et al.* [5] proposed that TcR1-bearing T cells are rich in the intestinal epithelium and mediate immunological surveillance of the epithelium. In this connection Bucy *et al.* [1] have reported that TcR1⁺ cells, most of them expressing a CD8 homologue, are preferentially located in the epithelium of the chicken intestine. These results suggest that the T-cell subsets in the epithelium of the gut-associated lymphoid organs of the chicken have a common origin and possibly play a local defensive role.

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Fig. 3a

Fig. 3b

Fig. 4a

Fig. 4b

Figs. 3a-b. Relative frequencies of T-cell subsets in the epithelium of postnatal chickens bursa. At all the stages, there are more TcR1⁺ cells than TcR2⁺ cells (a) ($P < 0.05$ at 5 and 15 weeks), and CT8⁺ cells is higher than CT4⁺ cells (b) ($P < 0.05$ from 5 to 15 weeks). Values are given as the mean $\pm$ standard error ($n=3$).

Figs. 4a-b. Relative frequencies of T-cell subsets in the lamina propria of the chickens bursa at various postnatal stages of development. In the lamina propria, TcR2⁺ cells (a) ($P < 0.05$ at 9w) and CT8⁺ cells (b) ($P < 0.05$ at 5 and 9 weeks) are predominant at all the stages rather than TcR1⁺ and CT4⁺ cells, respectively. Values are given as the mean $\pm$ standard error ($n=3$).

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