Immunohistochemical Investigations of Lymphocytes in the Lymphoid Organs of Cyclophosphamide Treated Chickens

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ABSTRACT. The localization of lymphocytes in the lymphoid organs of cyclophosphamide (Cy) treated chickens and untreated control chickens was compared immunohistochemically using a variety of monoclonal antibodies (CT3, 2-6, 11-39, TCR1, TCR2, TCR3, L22, 11G2, 3E8, B-4D-4, A-13). In the Harderian glands of Cy treated chickens, an increase of T cells was observed, though T cells were a few in untreated controls. These increased T cells consisted of CD4 positive or CD8 positive cells. Further, these T cells were stained with TCR2 or TCR3 antibody, and a small number of cells were stained with TCR1 antibody. In other lymphoid organs such as the bursa of Fabricius, thymus, spleen and cecal tonsils, B lymphocytes severely decreased or disappeared in Cy treated chickens, though no significant alteration in T cell distribution was observed.—Key words: cyclophosphamide, Harderian gland, immunohistochemistry.


Chickens have been used as experimental animals for the studies of the immune system, because chicken T and B cells mature at the separate organs, thymus and bursa of Fabricius, respectively, and these organs are easily manipulated. Recently a variety of monoclonal antibodies (mAbs) reactive with chicken lymphocyte surface markers have been produced, and comparative analysis of T and B cells using these monoclonal antibodies reveals that the biochemical characteristics of the identified molecules is highly conserved in birds and mammals [2].

Cyclophosphamide (Cy) is widely used to investigate the function of the avian immune system. Injections of Cy to newly hatched chickens affect the immune system, and eliminate bursal lymphocytes [7]. In this study, we investigated the effect of Cy on chicken lymphoid organs immunohistochemically using several mAbs for chicken lymphocytes.

mAbs used were as follows; CT3 (CD3 specific), TCR1 (y^TCR T cell specific), TCR2 (a^TCR T cell, V^B1-specific), TCR3 (a^TCR T cell, V^B2-specific), 2-6 (CD4 specific), 11-39 (CD8 specific), L22 (Bu-1a specific), 11G2 (Bu-1b specific), 3E8 (IgG specific), B-4D-4 (IgA specific) and A-13 (IgM specific). For the detection of Bu-1 antigens, a mixture of L22 and 11G2 was applied.

Line V chickens (MHC; B^15B^15), originally derived from University of Turku, Finland, and kept in our institute, were used. After hatching, chickens were intramuscularly injected with Cy (Shionogi & Co., Ltd., Osaka, Japan) at the dosage of 3 mg/head a day for 4 consecutive days. Uninjected chickens were served as controls. A total number of 9 CY treated and 9 untreated control chickens was euthanized at the ages of 5 weeks, 7 weeks and 6 months. Tissues of the Harderian gland, bursa of Fabricius, thymus, spleen, cecal tonsil were obtained. Fresh tissues were embedded in OCT compound (Miles Inc., U.S.A.), snap frozen in liquid nitrogen. The 4 µm frozen sections were fixed in acetone at 4°C for 10 min, and rehydrated in phosphate-buffered saline (PBS, pH 7.2). Tissue sections were first covered with 10% heat inactivated horse serum for 30 min. The sections were then incubated successively for 30 min each with the primary mAb, biotinylated anti-mouse IgG, 0.3% H_2O_2 in methanol, the avidin-biotin peroxidase complex (Vector Laboratories, Inc., U.S.A.), and stained with 3,3'-diaminobenzidine (0.2 mg/ml) and 0.01% H_2O_2. The sections were then counterstained with Mayer's hematoxylin (Merck, Germany).

In the interstitium of the Harderian glands of normal chickens, massive accumulation of lymphoid cells was observed at all ages. These lymphoid cells were IgG-, IgM- or IgA-positive (Fig. 1A). Only few lymphoid cells were positively stained with anti-CD3 antibody (Fig. 2A). These CD3 positive cells were also stained with anti-CD8 antibody, and to the lesser extent, stained with anti-CD4 antibody. Additionally, most of CD3 positive cells were positive for either chicken TCR2 or TCR3, and CD3 positive cells stained with anti-CD3 antibody were negligible.

On the other hand, in the Harderian glands of CY treated chickens, lymphoid cells decreased. Immunohistochemically a small number of cells were positive for chicken IgM, but were negative for chicken IgG and IgA (Fig. 1B). Instead, an increase of CD3 positive cells (Fig. 2B) was detected in all CY treated chickens. Most of these CD3 positive cells were also stained with anti-CD4 or anti-CD8 antibody. Furthermore these CD3 positive cells were predominantly stained with anti-TCR2 or anti-TCR3 antibody, and a few cells were positive for TCR1.

In other lymphoid organs such as the bursa of Fabricius, thymus, spleen and cecal tonsils, a severe decrease or disappearance of B lymphocytes was observed, though no significant alteration in tissue distribution of T cells was observed.

One of immunohistochemical features in CY treated chickens in this study is an increase in the relative proportion of T cells in the Harderian glands. These increased T cells were dominantly distributed in the interstitium of the Harderians.

In the Harderian gland, accumulating lymphoid cells which are mainly B cell lineage [1, 6] may play an important role in local immunity to produce antigen specific immunoglobulins [4, 5]. On the other hand, in CY

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treated chickens, remaining lymphoid cells were predominantly CD3 positive, and to the lesser extent, IgM positive cells, though IgG or IgA positive cells were never observed at the age ranged from 5 weeks to 6 months old.

The function of infiltrating T cells in the Harderian gland is not determined yet. One possibility is that these T cells may play some roles in local immunity instead of decreased B cells. Still, recently Cihak et al. [3] reported that V[b]1 TCR T cells play an important role in the production of IgA antibody in chickens. In this connection, it is interesting to study whether infiltrating T cells in Cy treated chickens have a function of immunoglobulin class switch as suggested in the other peripheral lymphoid organs such as the spleen and cecal tonsils [8]. To clarify true nature of these cells, further investigations are needed.

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