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Full Length Article

Detection of immunoglobulins containing plasma cells in the thymus, bursa of Fabricius and spleen of vaccinated broiler chickens with Newcastle disease virus vaccine

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Abstract Mobilization of immunoglobulins (Igs)-containing plasma cells (IgA, IgG and IgM) in the spleen, bursa of Fabricius and thymus was investigated in broiler chickens that were vaccinated with Newcastle disease virus (NDV) vaccine. In the thymus, the Igs-containing plasma cells were distributed in the cortex and medulla. Their frequency and distribution were higher at D_{14} and at D_{28}. The number of IgG- and IgM-positive cells was greater than IgA-positive cells in thymus. In the bursa of Fabricius, Igs-containing plasma cells were distributed beneath the capsules; within and around the bursal follicles. Their frequency of occurrence significantly peaked at D_{14} and at D_{28} in comparison to day-old chickens, and IgG-positive cells were significantly greater than the IgA- and IgM-positive cells in the bursa of vaccinated chickens. In the spleen, Igs-containing plasma cells were distributed in the white pulp, around the trabeculae, and in the periarterial lymphatic sheath. In this secondary lymphatic tissue, IgG- and IgM-positive cell numbers significantly greater

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than IgA-positive cells. In conclusion, mobilization of more Igs-positive cells in lymphoid tissues of broiler chickens is due to the effect of NDV vaccine as well as the advancement of age.

1. Introduction

Poultry production, especially chickens and ducks has attained an important place in the agricultural economy of Bangladesh both through contribution to gross domestic product (GDP) and employment, especially in urban and pre-urban areas. Newcastle disease (ND) is the most common and harmful disease for commercial broilers. In addition, ND causes serious outbreaks in commercial layer and parent stocks every year and results in huge losses for the poultry industry. ND leads to necrosis, infiltration of heterophils and heterophilic changes in lymphoid organs [1].

Most living beings manage not only to survive but indeed thrive in a potentially hostile milieu, with seemingly little effort. This freedom from disease is dependent on the existence of a complex and highly sophisticated defense system called lymphoid system [2] which start to develop at embryonic day (ED) 10 in broilers [3,4].The lymphoid system of fowl is consisting of unique organs and divided into two morphologically and functionally distinct components [5]. The lymphoid tissues have an independent phylogenetic origin, their function being to react to foreign antigens by producing antibodies, thereby providing ''adaptive immunity''. An obvious characteristic of the lymphatic tissues of mammals and birds is that they are densely populated with lymphocytes. This is because they are involved in the lymphocytes production, immune responses or both of these occurring at the same time [6]. When lymphocytes arrive in the lymphoid tissues or organs they become plasma cells and begin to synthesize immunoglobulins. The plasma cells-containing different classes of immunoglobulins (Igs) are distributed throughout the lymphoid tissues, [7] including Harderian gland of broiler chickens [8].

Chickens have been used as experimental animals for studies of the immune system, because chicken T and B cells mature in the thymus and bursa of Fabricius, respectively, and because these organs are easily manipulated. The development, differentiation, distribution and function of immune cells in these tissues, including the spleen of chicken, have been investigated using a wide variety of methodologies: surgical thymectomy, bursectomy, X-ray irradiation, and the colloidal carbon uptake method, as well as the administration of hormones, drugs, vitamins and minerals [9–14]. In the present study, we used NDV vaccine via both ocular and oral routes to study the frequency and distribution of Igs-containing plasma cells in the thymus, bursa of Fabricius and spleen of broiler chickens.

2. Materials and methods

The experiment was carried out in the Department of Anatomy and Histology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. Part of the research was also conducted in the Laboratory of Anatomy and Histology, Faculty of Veterinary Medicine, University Malaysia Kelantan, Malaysia. The following materials and methodologies were followed in the present experiment:

2.1. Chickens

A total of 32 day-old ''Cob-500'' broiler chickens of both sexes were purchased from Kazi Farm Ltd. Mymensingh, Bangladesh. The chickens had no visible developmental disorders or detectable diseases that might influence the distribution of lymphocytes and plasma cells.

2.2. Management

The chickens were reared in a litter system in a poultry shed at the Department of Poultry Science, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh. All the chickens were provided with broiler feed and mineral water *ad libitum*. Poultry shed bio-security and brooding management were both strictly maintained, as were optimum temperature, lighting and ventilation in the brooder.

2.3. Vaccines

The live attenuated NDV vaccine used in this study was the product of Mohakhali Research Centre, Directorate of Livestock Services, Dhaka, Bangladesh. The recommended dose is one drop of vaccine per eye in between one to 3-days-old chicken, with a booster dose usually given between 12 and 21 days of age.

2.4. Experimental design

The chickens were grouped into control and treatment groups. Chickens in the control group ($n = 16$) were given a drop of distilled water ocularly at 06:00 on D_1. From this group 8 chickens were killed at 18:00 on the same day (D_1), and the remaining 8 chickens were sacrificed on D_{10} at 18:00 to ascertain the mobilization of immune cells. Chickens in the treatment group ($n = 16$) were given NDV vaccine ocularly on D_3 at 06:00 with a booster dose of the same vaccine being administered orally on D_{13} at 06:00. The thymus, bursa of Fabricius and spleen were collected from these chickens on D_{14} and D_{28} after sacrificing the chickens (8 broilers per group) through cervical subluxation, and specimens were fixed in Bouin's fluid or ice cold periodate lysine paraformaldehyde (PLP) for histological and immunohistochemical study.

2.5. Antibodies

The following antibodies were used for detecting Igs-positive cells:normal rabbit serum (Biosource international, Inc., Camarillo, CA, USA), goat anti-chicken IgA (Bethyl Laboratories, Montgomery, TX, USA), goat anti-chicken

IgG (Bethyl Laboratories), goat anti-chicken IgM (Bethyl Laboratories) and HRP-conjugated rabbit anti-goat IgG (Bethyl Laboratories).

2.6. Immunohistochemistry

The indirect immunoperoxidase method was performed to study the distribution pattern and frequencies of Igs-positive cells in the bursa of Fabricius, thymus, and spleen, of control and vaccinated broiler chickens. The tissues from bursa of Fabricius, thymus and spleen were fixed in Bouin's fluid or PLP, dehydrated in a series of graded alcohol, cleared in xylene, and embedded in paraffin. Paraffin sections of 6 μm thickness were immunostained by the indirect immunoperoxidase method as described previously [14].

2.7. Histoplanimetry

The Igs-containing plasma cells were counted in 20 fields of the histological sections using a light microscope at a magnification of 400× where Igs-plasma cells were diffusely distributed and their relative frequency per focus was calculated according to the point count method [16].

2.8. Statistical analysis

The numbers of Ig-containing plasma cells and their variation in numbers in the thymus, bursa of Fabricius and spleen were compared (randomized block design) among broilers of various stages, and data were evaluated by student's *t*-test [17].

3. Results and discussion

Frequency and distribution of Igs-containing plasma cells (IgA, IgG and IgM) in the lymphoid tissues of broiler chickens:

3.1. Thymus

In the thymus, Igs-containing plasma cells were distributed in the cortex and medulla (Fig. 1a–c). The frequency of Igs-containing plasma cells was statistically greater ($P < 0.05$) in the thymus at D_{14} and D_{28} in comparison to the day-old chickens (Figs. 4–6). When comparing frequency of occurrence of different Igs-containing plasma cells, it was found that IgG- and IgM-positive cells were greater ($P < 0.05$) at D_{14} and D_{28} ages. In the present study, the population of Igs-containing plasma cells and their infiltration to the lymphoid organs due to inoculations of NDV vaccine were clearly observed. These dynamic changes in the frequency and distribution of Igs-containing plasma cells were also observed in the Harderian gland, cecal tonsil and trachea of our previous studies [14]. The thymus is responsible for the production of T-lymphocytes subsets in the chicken [18] not Igs-containing plasma cells, however existence of statistically higher IgG- and IgM-positive cells in the thymus of the present study indicates that the B lymphocytes infiltrated in the thymus via blood circulation and differentiated into Igs-containing plasma cell for local defensive function as T-lymphocytes subpopulation do in the bursa of Fabricius [19], which is the homing organ for B lymphocytes.

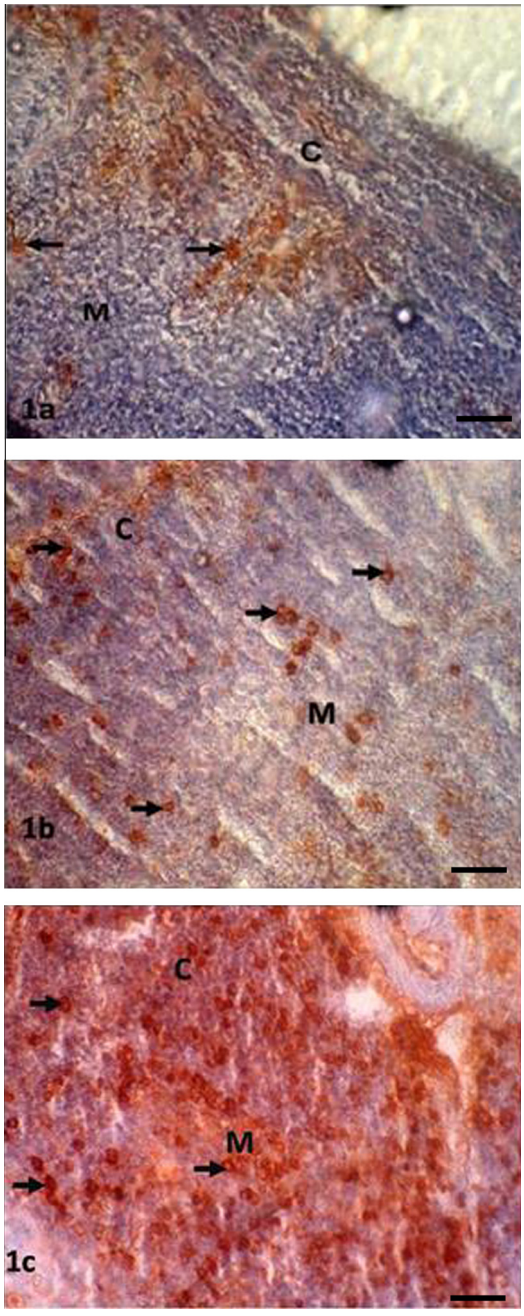

Figure 1 (a–c) Immunostained section of chicken thymus of broiler chickens at D_1(**a**), D_{14}(**b**), and D_{28}(**c**) showing IgM-positive cells (arrows) in cortex (C) and medulla (M). IgM-positive cells are numerous at D_{14} and at D_{28}. Scale bar = 50 μm.

3.2. Bursa of Fabricius

In the bursa of Fabricius, Igs-containing plasma cells were found principally beneath the capsule; in the cortex, and medulla of the bursal follicles; and around the bursal follicles (Fig. 2a and b). The frequency of Igs-containing plasma cells in this gland was greater at D_{14} and at D_{28} in comparison to day-old chickens (Figs. 4–6). When comparing the data, it was found that, the frequency of IgG-positive cells was statistically higher ($P < 0.01$) at D_{28}. In contrast, IgM-positive cells

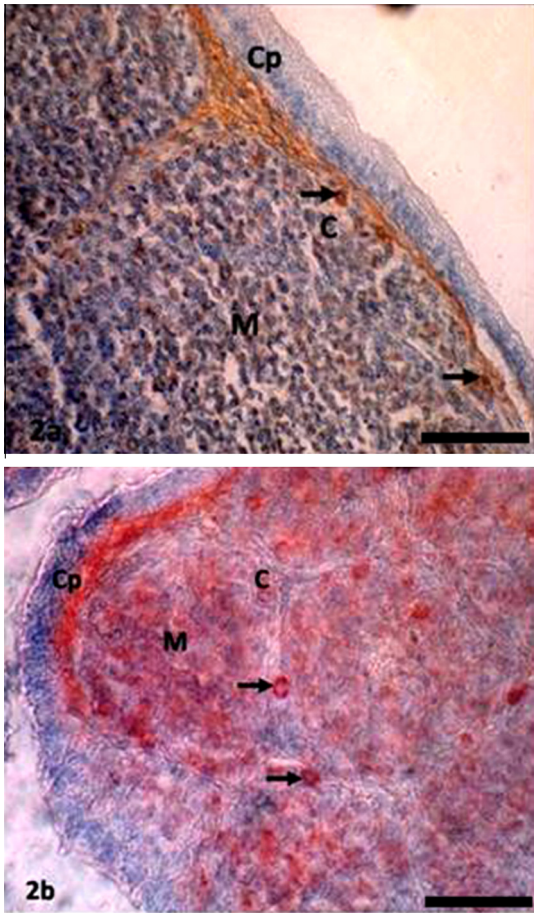

Figure 2 (a and b) Immunostained sections of bursa of Fabricius of broiler chickens at D_1**(a)** and D_{14}**(b)** showing IgA-positive cells (a) and IgG-positive-cells (b) (arrows) in the cortex (C), medulla (M), and beneath the capsule (CP) of the bursal follicle. The immunopositive cells are more numerous at D_{14} than day-old-chickens. Scale bar = 50 μm.

were insignificantly greater than IgA-and IgG-positive cells at D_1 and at D_{14}. The findings of our present study varied greatly [20]. Honjo et al. [15] reported that most of the bursal lymphoid cells of 4 week old inbred line P chickens were IgM positive. This variation at the same age is possibly due to the strain differences of chickens used in the present study. In the vaccinated chickens of the present study Igs-containing plasma cells were sharply increased from the D_1 chickens and peaked from D_{14} to D_{28} and this might be due to immunomodulation of the vaccine.

3.3. Spleen

In the spleen, Igs-containing plasma cells were numerous in the white pulp (Fig. 3a–c), around the trabeculae, around the central artery and in the periarterial lymphatic sheath. These distribution patterns of Igs-containing plasma cells in the vaccinated chickens were similar to our previous study [13], where we used vitamins and minerals to observe the distribution pattern of Igs-containing plasma cells. A similar distribution pattern of Igs-containing plasma cells was also observed in the spleen of the control group in the present study. The frequency and distribution of IgG-positive cells were

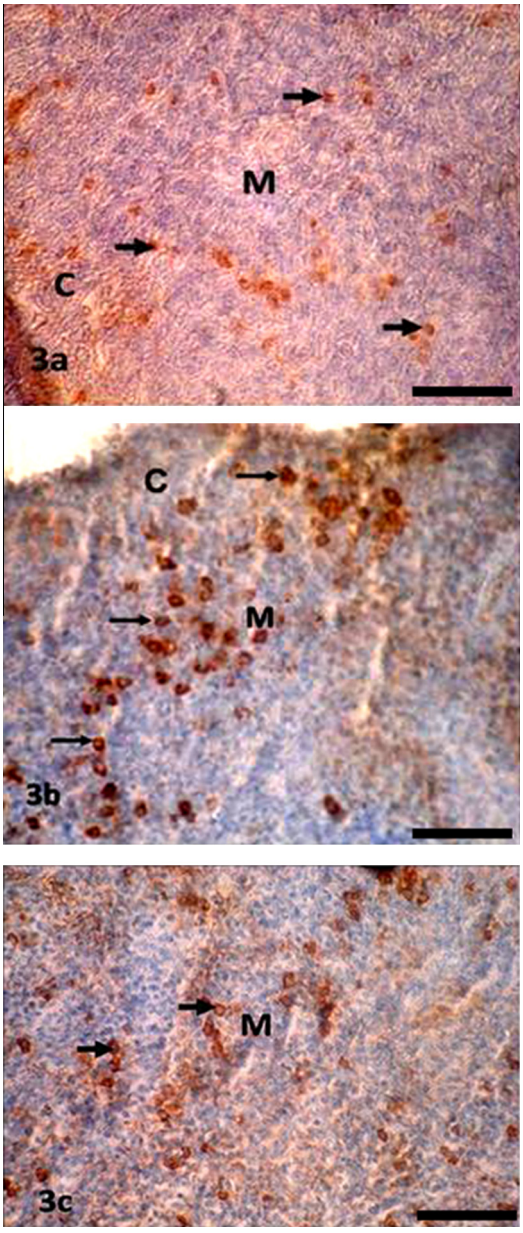

Figure 3 (a–c) Immunostained sections of spleens of chickens at D_1**(a)**, D_{14}**(b)** and D_{28}**(c)** showing IgA-positive cells (a), IgG-positive cells (b) and IgM-positive cells (c) (arrows) in the white pulp of the spleen. In the spleen, immunopositive cells are more numerous at D_{14} and D_{28}. Scale bar = 50 μm.

statistically greater ($P < 0.01$) at D_{14} (Fig. 5); and IgA- and IgM-positive cells were higher at D_{28} (Figs. 4 and 6). When comparing the data of Igs-containing plasma cells in this gland, it was observed that, both IgG- and IgM-positive cells were statistically higher ($P < 0.01$–0.05) than the IgA-positive cells throughout the period of postnatal development. This finding was in agreement with our previous statement that the NDV vaccine initiates mobilization of similar types of immune cells in the Harderian gland of broiler chickens [14].

Chickens were inoculated with NDV vaccine at D_3, and the booster dose was given at D_{13} in the present study. We compared the data of Igs-containing plasma cells between vaccinated chickens and control up to D_{10}, and it was found

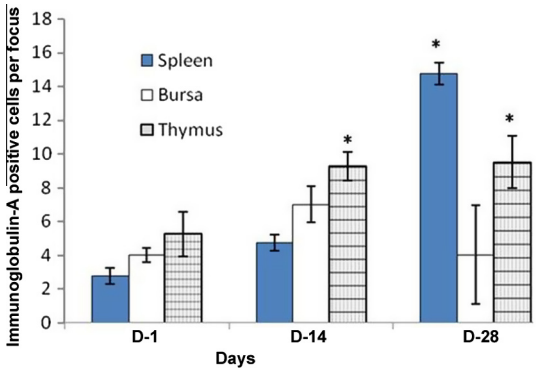

Figure 4 The frequency of IgA positive cells in the thymus, bursa of Fabricius and spleen of broiler chickens from D_1 to D_{28}. IgA-positive cells are greater at D_{14} and D_{28} chickens in than in D_1. The frequency of these immune cells is significantly greater ($P < 0.01$) in the spleen, followed by the thymus and bursa of Fabricius at D_{28} of development. The values are given as the mean ± standard error ($n = 8$).

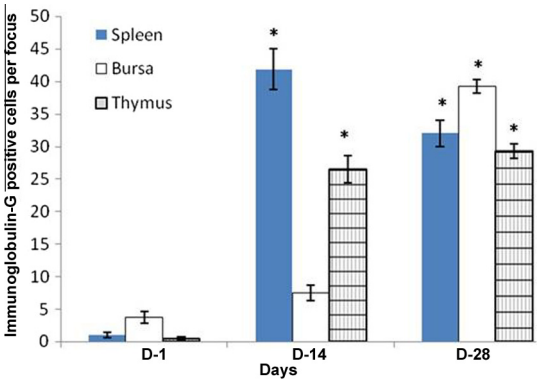

Figure 5 The frequency of IgG-positive cells in the thymus, bursa of Fabricius and spleen of chickens during their postnatal growth from D_1 to D_{28}. IgG-positive cells are more frequent in D_{14} and D_{28} chickens than in comparison to D_1 chickens. The frequency of these cells are significantly greater ($P < 0.01$) in the spleen, followed by the thymus and bursa of Fabricius at D_{14} in chickens, whereas at D_{28}, IgG cells are found more numerous in the bursa of Fabricius. The values are given as the mean ± standard error ($n = 8$).

that the number of Igs-containing plasma cells were significantly greater in vaccinated chickens than in control. In the present study, it was observed that the frequency and distribution of IgA-, IgG- and IgM-positive cells were more in D_{14} and D_{28} groups of vaccinated chickens than the chickens of D_1. This finding was similar to the report of Salam et al. [20] and Solcan et al. [21]. Among the Igs-containing plasma cells, the number of IgG- and IgM-positive cells was significantly greater at D_{14} and D_{28} groups of chickens. This finding is in partial agreement with the results of Bienenstock et al. [22] and Ohshima et al. [23], who reported that both IgA- and IgG-positive cells formed the bulk of the lymphoid cell

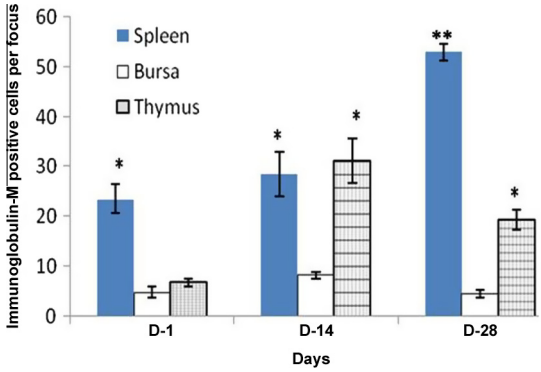

Figure 6 Frequency of IgM-positive cells in the thymus, bursa of Fabricius and spleen of chickens during their postnatal growth from D_1 to D_{28}. The frequency of IgM-positive cells is greater at D_{14} and D_{28}, and in the spleen these cells are significantly greater ($P < 0.01$–0.05) at D_1 and D_{28} followed by the thymus and bursa of Fabricius. However, at D_{14} these immune cells are more numerous in the bursa of Fabricius, but the difference is not significant. The values are given as the mean ± standard error ($n = 8$).

population in the early stages of life and that IgA-positive cells were predominant in the later stages of life.

IgG and IgM cells protect the body from infection are found in the lymphoid organs, whereas IgA cells are found in the secretory organs [24]. The frequency of these cells and the antibody titre in the serum increased with the NDV vaccine alone [25] or perform synergistic effect with immune adjuvant [26]. In the present study, the number of IgG- and IgM-positive cells was statistically higher than IgA-positive cells in the thymus and bursa of Fabricius. In contrast to the Harderian gland of the duck [27] and chicken [8]; and to the mucosa of the gastrointestinal tract of chickens [7], IgA-positive cells were significantly greater. This observation from avian species was similar to the mammalian context, particularly the rat [24]. This finding suggests that the frequency and distribution of Igs-containing plasma cells are in similar in avian and mammals despite belonging to different phyla.

4. Conclusion

In the present study, immunoglobulins-positive cells were detected in the lymphoid organs using immunohistochemistry. The results demonstrate that IgG- and IgM-containing plasma cells are abundantly found in the thymus, bursa of Fabricius and spleen of broilers chickens. Cells numbers were statistically greater at D_{14} and at D_{28} due to an immune response resulting from the NDV vaccine as well as the advancement of age.

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