Journal of Animal and Veterinary Advances 9 (4): 751-755, 2010
ISSN: 1680-5593

Effects of Photoperiod on Number of Mast Cells in Lymphoid Organs of the Japanese Quail (*Coturnix coturnix japonica*)

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Abstract: The distribution of Mast Cells (MCs) was studied in the lymphoid organs of 7, 14, 21 and 30 days old quails (*Coturnix coturnix Japonica*) kept in different photoperiods using light microscopy histochemical techniques. The distribution of MCs was determined in different age groups housed in continuous light (23L:1D) or in a light-dark regimen (18L: 6D). Tissue samples were obtained under deep anesthesia from birds in the four age groups, fixed in Mota's fixative (basic lead acetate) for 24 h and embedded in paraffin. Six micrometre-thick sections were stained with 0.5% toluidine blue and the number of MCs counted under the microscope. The numbers of MCs were significantly different between both age and light treatment groups. The number of mast cells was significantly lower ($p<0.05$) in birds exposed to continuous light (23L:1D) than in birds exposed to a shorter light period (18L:6D). Photoperiod was concluded to influence mast cell numbers in the lymphoid organs of the Japanese quail and thus the immune response of the birds.

Key words: Lymphoid organs, photoperiods, treatment group, birds, MCs, Turkey

INTRODUCTION

Mast Cells (MC) develop from bone marrow-derived cells that circulate in the blood as mononuclear agranular cells (Kitamura *et al*., 1978; Kirshenbaum *et al*., 1999). Their differentiation and maturation occur in peripheral tissues and are most likely regulated by local microenvironmental factors, such as growth factors and cytokines, in particular the c-kit ligand (Gurish and Austen, 2001). Two types of mast cells, connective tissue mast cells and mucosal mast cells have been defined in birds and rodents based on different histological, functional, compositional and pharmacological regulatory properties (Karaca *et al*., 2006; Irani *et al*., 1986).

Mast cells have important regulatory functions in inflammation (Church and Levi-Schaffer, 1997; Mekori and Metcalfe, 1999). Mast cells are derived from hematopoietic precursors and represent critical effector cells in allergic diseases and other IgE-dependent responses. In addition, these cells appear to be involved in other pathophysiological processes including delayed-type hypersensitivity, wound healing, fibrosis and neuroimmunological disorders.

The neuroendocrine system plays a critical role in homeostatic regulation of the immune response (Kliger *et al*., 2000). The neuroendocrine functions of the pineal gland are closely linked to the 24 h light:dark cycle (Klein, 1979) and these pineal-immune interactions are also thought to have a temporal component. Evidence of effects of the pineal gland on photoperiodic changes in the immune system has been published for birds and mammals (Kliger *et al*., 2000; Majewski *et al*., 2005; McNulty *et al*., 1990). For example, pinealectomy reduces cellular and humoral immune responses in Japanese quail, but these are improved to normal levels by melatonin replacement (Moore *et al*., 2002). Melatonin restores the impaired T-helper cell activity in immunosuppressed mice and enhances antigen presentation to T cells through splenic macrophages (Kliger *et al*., 2000; Pioli *et al*., 1993). The objectives of the present study were to characterize the distribution of mast cells in the lymphoid organs (thymus, spleen and bursa of Fabricius) of quail and to determine the effects of different photoperiod regimens and bird age on these mast cell distributions.

MATERIALS AND METHODS

Fertilized eggs of Japanese quail (*Coturnix coturnix Japonica*) were obtained locally, incubated in a forced draft poultry incubator with 75% relative humidity at 37.2±0.2°C and hatched under appropriate conditions. Four groups of 6 birds each were chosen: 7, 14, 21 and

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30 days post-hatching. The newly hatched quail (= 0 days old) were housed in floor pens and were randomly divided into one of two different photoperiod treatments: a light period of 23 h light:1 h darkness or 18 h light: 6 h darkness. Food and water were provided *ad libitum* throughout the experiment. At the end of the experiment, the organs (thymus, spleen and bursa of Fabricius) were removed under pentobarbital anesthesia (50 mg kg^{-1} b.w., i.p.). When used for histological techniques, tissue fragments were fixed in Mota's basic lead acetate (BLA, 24 h) and then embedded in paraffin. For mast cell counts and identification, serial 6 μm thick cross-sections were taken from prepared blocks and stained with 0.5% toluidine blue (Merck, CI No. 52040) solution, which was prepared in Mac Ilvaine's citric acid disodium phosphate buffer (pH 0.5).

Microscopic examination: Microscopic examination was carried out under a magnification of 400x and the counts of mast cells were determined per square millimeter using a standardized ocular grid. The density and distribution of MCs were examined in the sections stained with toluidine blue. Tissue sections were examined under light microscopy and the number of mast cells counted in random high-power fields using a Nikon Optiphot 2 light microscope incorporating a square graticule in the eyepiece (eyepiece x10, objective x40, total side length of 0.225 mm).

Mast cell density was assessed by counting the number of cells in 100 high power fields in lymphoid tissue preparations of each group. The MC density in each site was calculated and recorded as MC numbers mm^{-2}. All the results were expressed as mean±SE.

Statistical analysis: All values are expressed as mean±SD. Statistical analysis of data was performed using one-way analysis of variance and Tukey's post-test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The distribution of the Mast Cells (MCs) is summarized in Table 1 and Fig. 1-6. The histochemical study of tissues stained with the toluidine blue technique showed that regardless of age or light cycle, mast cells were present in the lymph follicles, spleen and bursa of Fabricius. The mean MC density showed variations both between the different organs and between different tissue locations within one organ. Numerous MCs were observed in the blood vessels around the thymus, spleen and bursa of Fabricius and in the subepithelial loose connective tissues in the bursa of Fabricius.

Table 1: Effect of different photoperiods on mast cell numbers in the lymphoid organs of quail aged 7, 14, 21 and 30 days

	Thymus			
Age (days)	Cortex	Medulla	Spleen	B. Fabricius
Light (23:1)				
7	23±7^{b}	54±21^{c}	72±9^{b}	48±11^{b}
14	31±15^{b}	81±26^{a}	76±14^{b}	73±16^{a}
21	28±6^{b}	74±19^{b}	121±28^{a}	50±14^{b}
30	40±22^{a}	65±8^{b}	112±26^{a}	40±10^{c}
Light (18:6)				
7	35±7^{c}	69±9^{c}	81±12^{c}	64±15^{b}
14	44±15^{b}	103±25^{a}	97±11^{c}	85±21^{a}
21	39±9^{c}	78±14^{b}	135±18^{b}	67±10^{b}
30	51±15^{a}	73±16^{c}	157±25^{a}	52±16^{c}

Values are Mean±SE, n = 6, Means followed by same letters in a column are not significantly different at 5% level of significance

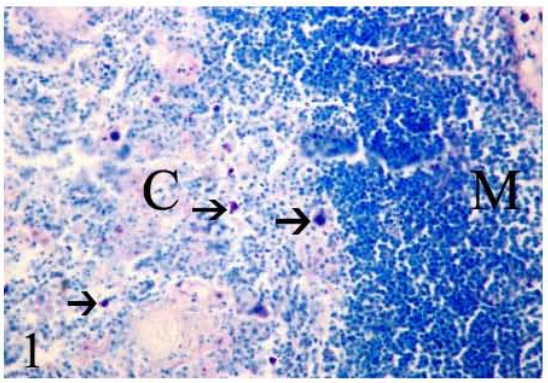

Fig. 1: Section of thymus from a 14 days old quail (23:1 light-darkness). C: Cortex; M: Medulla; Arrows: Mast cells. Toluidine blue, X360

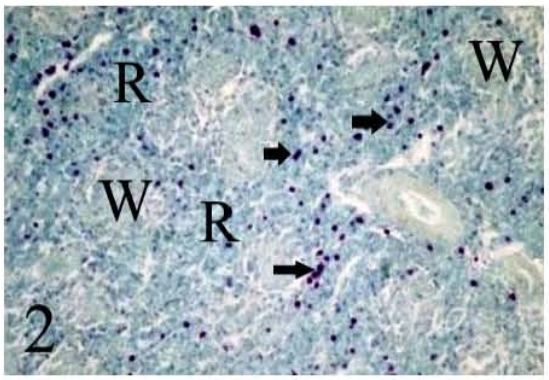

Fig. 2: Section of spleen from a 14 days old quail (18:6 light darkness). R: Red pulp; W: White pulp; Arrows: Mast cells. Toluidine blue, X360

Thymus: Mast cell density was higher in the medullar region of the thymus than in the cortical region. In all groups, the thymus capsule and subcapsular area exhibited several MC sparsely distributed among the numerous fibroblasts and connective tissue fibrils. In the 14 days old and 18:6 light-darkness quail, the numbers of

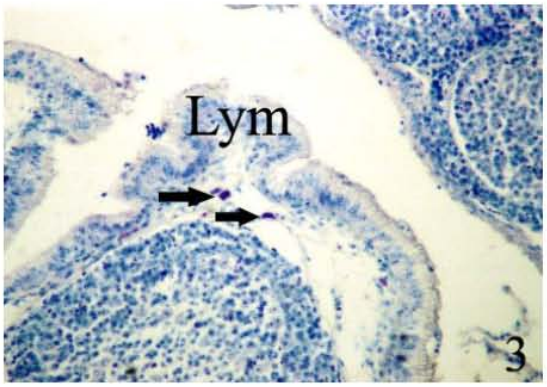

Fig. 3: Section of bursa of Fabricius from a 7 days old quail (18:6 light-darkness). L: Lamina epitelialis; Lym: Lymph follicles; Arrows: Mast cells. Toluidine blue, X360

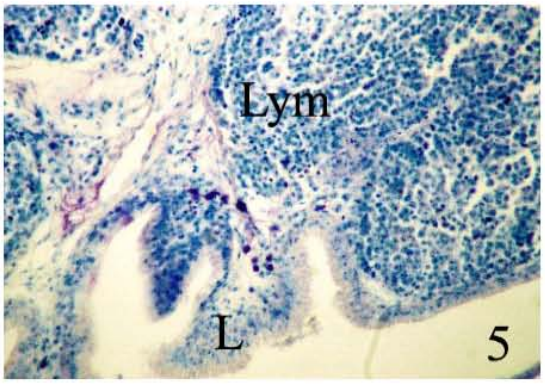

Fig. 5: Section of bursa of Fabricius from a 14 days old quail (23:1 light-darkness). L: Lamina epitelialis; Lym: Lymph follicles; Arrows: Mast cells. Toluidine blue, X360

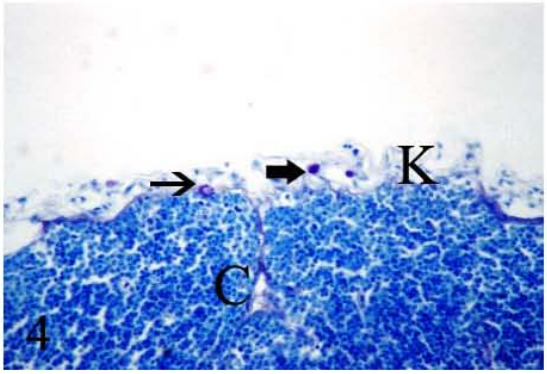

Fig. 4: Section of thymus from a 30 days old quail (23:1 light-darkness). C: Cortex; K: capsule; Arrows: Mast cells. Toluidine blue, X360

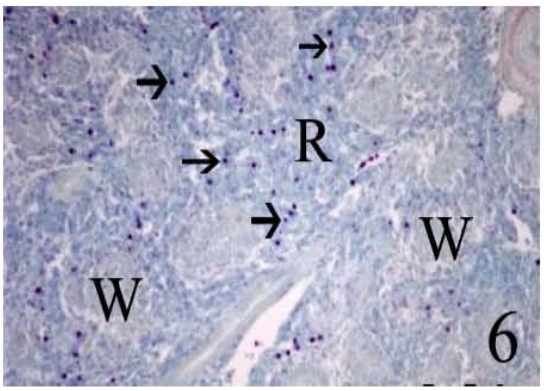

Fig. 6: Section of spleen from a 21 days old quail (23:1 light-darkness). R: Red pulp; W: White pulp; Arrows: Mast cells. Toluidine blue, X360

MCs were higher in the medullar region and there were significantly higher numbers of MCs in the thymus ($p<0.05$) than for the other age groups (Fig. 1 and 4).

Spleen: In the spleen, MCs were not observed in the white pulp. The pattern of MC distribution was different, because they were particularly localized in sinuses of red pulp, where they were found predominantly around blood vessels. In the spleen, the highest mast cell numbers were noted in the 21 days old quail, regardless of the light regimen. The number of MCs was significantly higher in the spleen of 21 and 30 days old quail than in the 7 and 14 days old birds for both photoperiods (23:1 and 18:6 light-darkness) (Fig. 2. and 6).

Bursa of Fabricius: Mast cells were frequently observed between the follicles and subcapsular area, whereas MCs were not observed in the lymphoid follicles. In the inter follicle area, toluidine blue staining analysis revealed that the distribution pattern of the MCs was different from that in the subcapsular area, with higher density in the subcapsular area than in the interfollicle area. In the bursa of Fabricius of the 14 and 21 days old birds, the MC counts were significantly higher than those of the 7 and 30 days old birds for both photoperiods (Fig. 3 and 5).

This study examined whether, the distribution of MCs in quail thymus, bursa of Fabricius and spleen undergoes post-natal age and light-dependent changes. In literature search, no study on distribution and location of mast cells within lymphoid tissues in quail was met on photoperiod dependent changes. Therefore, the findings were compared with the data belonging other species in literature.

Mast cell density varied and showed unique localization patterns within some organs. These results confirm and strengthen earlier observations that mast cells are found with varying density in a number of chicken lymphoid organs (Karaca *et al*., 2006;

Crivellato *et al*., 2005). Previous histochemical, immunocytochemical and ultrastructural studies have revealed MCs in the thymus, spleen and bursa of Fabricius of mammalian and avian species (Crivellato *et al*., 2005; Bigaj *et al*., 1991; Karaca *et al*., 2006). Karaca *et al*. (2006) reported the existence of mast cells in chicken lymphoid tissues (thymus, spleen and bursa of Fabricius) and changes in MCs related to ageing. That study showed that mast cells were present in higher numbers in the medullar region than in the cortical region of the thymus. However, mast cells were also observed in higher numbers in the bursa of Fabricius of 7 days old birds than that of 0, 21, 30 and 120 days old birds. In the thymus, however, MC, these cells counts were higher in the 21 days old birds than in other age groups. In the present study, the mast cell population in lymphoid tissues increased with light restriction, with the 21 days old quail having a smaller increase than the other age groups. In addition, MC density was higher in the medullar region of the thymus than in the cortical region, although this might vary with greater age.

This distribution pattern places mast cells in areas commonly exposed to parasites and other pathogens, including environmental antigens, which might come into contact with skin or mucosal surfaces (Galli, 1993). Mast cell subtypes can be distinguished by their histochemical properties (Enerback, 1986), reactivity to secretagogues (Shanahan *et al*., 1985), growth factor dependency (Hamaguchi *et al*., 1987) and type of granule proteases (Noviana *et al*., 2004).

Kliger *et al*. (2000) suggested that an intermittent photoperiod regimen enhances splenic immune functions of broiler chickens compared with constant lighting, with photoperiod having a direct effect on mitogen-induced splenocyte proliferation and the relative proportions of splenocyte populations, while also enhancing the sensitivity of lymphocytes to melatonin stimulation *in vitro*. However, Konakchieva *et al*. (1995) suggested that melatonin *in vitro* decreases human lymphocyte proliferation. Kliger *et al*. (2000) and Dobrowsolska and Gromadzka-Ostrowska (1984) suggest that increasing periods of darkness stimulate mitogen-induced splenocyte proliferation. Colombo *et al*. (1992) found that spleen cells obtained at night have a high sensitivity to melatonin. Mahmoud *et al*. (1994) reported that photoperiod influences thymus morphology in rats and suggested an involvement by melatonin.

Baccari *et al*. (1991) showed that mast cell numbers in the Harderian gland of the green frog (*Rana esculenta*) vary during the year, reaching maximum levels during the winter months. Significant increases in MC numbers have also been found in the small intestine and lung of the hedgehog (Suomalainen and Harma, 1951; Harma and Soumalainen, 1951) and in the duodenum of the bat (Smith *et al*., 1954 a, b) during hibernation. In the present study, MCs were significantly increased in the lymphoid organs with 18:6 light-darkness compared with 23:1 light-darkness. Similarly to the mammalian literature, recent reports in birds have suggested a close connection between melatonin and immune response, with exogenous melatonin being able to counteract the immunos-uppressive effects of constant light and pinealectomy (Moore and Siopes, 2000; Moore *et al*., 2002). It has been suggested that melatonin also acts as a mediator of environmental information in the bird, which can be transmitted quickly to the immune system for an appropriate response. As in mammals, this may be particularly useful for communication regarding changes in season and stress (Moore and Siopes, 2003; Nelson and Demas, 1996).

CONCLUSION

The results indicate that photoperiod regimen affects the distribution of mast cells in the lymphoid organs of quail and thus, plays an important role in affecting the immune response. These results further clarify the role of light in modulating immune functions in quail.

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