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(Course of Applied Animal Science)

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発達に対する sIgM 誘導因子の役割

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Role of the Surface sIgM-Inducing Factor in Development of B Lymphocytes in Chicken Embryonic Bursa of Fabricius

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The bursa of Fabricius plays essential roles in the establishment of immune functions of avian species as a primary site for differentiation and proliferation of B lymphocytes. The bursa of chick embryos is colonized by lymphoid cell precursors only between the days 7th of embryogenesis (E7) and E14. Susceptibility to the sIgM-inducing factor may fluctuate in bursal lymphoid cells during the lymphoid precursor cell-receptive period. In the present study, the dynamic changes in the sIgM-positive ratio and responsiveness to sIgM-inducing factor were examined in lymphoid cells sampled from the bursa during the B precursor cell-receptive period (E10 to E13) and findings suggest that responsiveness to sIgM-inducing factor varies with the development of the chick embryos. E11 is suggested to be a critical stage of B-lymphocytegenesis in the bursa of chick embryos.

Key words : bursa of Fabricius, B lymphocytegenesis, sIgM-inducing factor

Introduction

Bursa-derived humoral factors involved in B cell development have been reported in chickens. Bursal extract induces Bu-1 alloantigen expression on the chicken B cell surface²⁾. Audhya *et al*¹⁾ isolated a bursa-derived low molecular weight peptide which elevated the cAMP level in a human B lymphoma cell line and they termed it Bursin. Bursin also accelerates the expression of Bu-1 antigen on bursocytes and the diversification of immunoglobulin repertoires¹¹⁾. Serum-supplemented culture supernatant of bursal epithelium elevated the Ia antigen-positive rate of chicken B lymphocytes³⁾. Recently, a factor which induced surface immunoglobulin M (sIgM) expression on bursal lymphoid cells of chick embryos has been reported in the supernatant of bursal epithelial cultures⁵⁾. The factor is supposed to be important in B cell development in the bursa because sIgM functions as the antigen receptor on B lymphocytes.

The bursa of chick embryos is colonized by lymphoid cell precursors between the 7th day of embryogenesis (E7) and E14. The receptive period for lymphoid precursors may be a critical period in which lymphoid cells in the bursa differentiate vigorously. IgM has been reported to be first evident both in the cytoplasm and on the surface of the bursal lymphoid cells around E12^{7,14)}. Thus it is useful to investigate dynamic changes in the sIgM-positive rate and responsiveness to lymphocyte-differentiating factors of bursal lymphoid cells in this

period. In the present study, changes in the sIgM-positive ratio and susceptibility to sIgM-inducing factor in bursal lymphoid cells were examined during lymphoid cell-receptive period of the bursa (E10 to E13).

Materials and Methods

Primary culture of bursal epithelial cells and preparation of sIgM-inducing factor

Bursal cells collected from 14-day-old chick embryos of White Leghorn Shaver strain were cultured at 39°C under 5% CO₂ in air as previous report⁴⁾. The cultured cells were maintained until 5 months without any apparent morphological abnormalities⁴⁾. The supernatant was recovered from the cultures that had been incubated for 3 weeks. The factor which induces IgM expression on the surface of immature bursocytes of chick embryos was prepared by gel filtration chromatography and HPLC as reported previously⁵⁾.

Bursal lymphoid cell preparation and counting of sIgM-positive cells

The bursae were sampled from chick embryos of the White Leghorn Shaver strain between E10 and E13. Lymphoid cells (bursocytes) were sampled from the bursae as described previously⁵⁾. The purity of the bursocytes in the cell suspension, determined by Natt and Herrick staining⁹⁾ was above 85%. Viability of the cells

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measured by trypan-blue exclusion test was about 70%. Rates of sIgM-positive cells were estimated by indirect immunofluorescence using goat anti-chicken μ chain monoclonal antibody (Bethyl Laboratory Inc., USA) and rabbit anti-goat IgG-FITC-conjugated antibody (Vector Laboratories, Inc., USA) in bursocytes incubated with 0.1 ml of the HPLC-fraction containing sIgM-inducing factor⁵⁾ for 0, 24, 48 and 96 hours at 39°C under 5% CO₂ in air. Surface IgM positive cells in bursocytes incubated with the fraction containing sIgM-inducing factor were calculated. The rates in bursocytes incubated with culture medium were used as control values.

Statistical analysis

Six to 7 samples were used in each experimental group. Bursocytes collected from 10 to 20 chick embryos were pooled for each sample. Student's t-test was used to determine the significance of differences between different age chick embryos. Significance was taken at the 5% probability level. All results are given as means and standard errors.

Results

Surface IgM-positive cells were found in the bursa at E10, although the rate was low ($6.3 \pm 0.6\%$) (Fig. 1). The rate was significantly elevated ($P < 0.05$) at E11 ($12.7 \pm 1.9\%$), E12 (13.5 ± 1.3), E13 (11.7 ± 0.8) and E14

(12.8 ± 0.3) (Fig. 1). There were no significant differences among the values at E11, E12, E13 and E14.

Surface IgM-inducing factor increased the sIgM-positive rate in bursal lymphoid cells sampled from E10 to E13 (Fig. 2-A, B, C and D). Figure 2-A shows the rates of sIgM-positive cells in bursocytes sampled from chick embryos at E10. The positive rates were increased by incubating the bursocytes with sIgM-inducing factor. There were significant differences between the positive

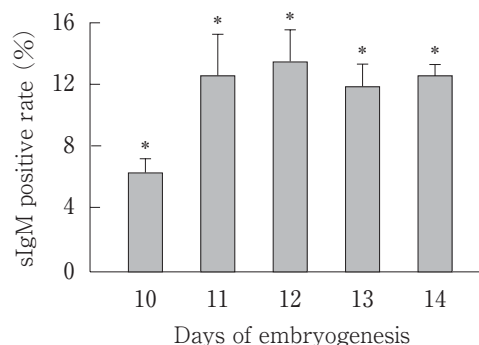


Fig. 1 Surface IgM-positive cell ratio in the bursal lymphoid cells sampled from chick embryos on the 10th day (E10) to 14th day of embryogenesis (E14).

The cells were stained with anti-chicken IgM antibody immediately after sampling. * = significantly different from the value at E10 ($p < 0.05$).

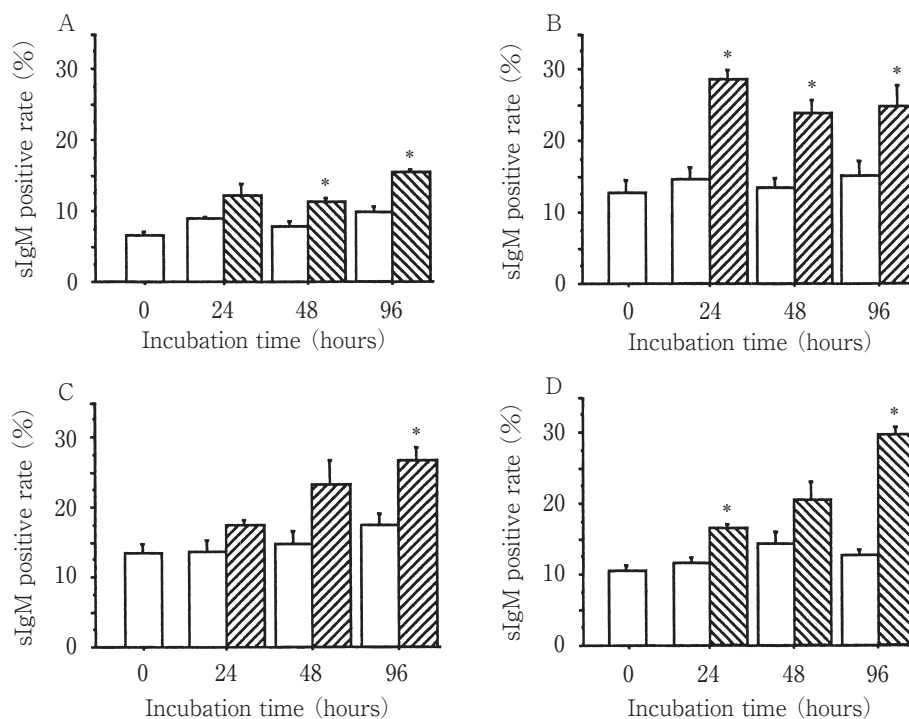


Fig. 2 Effects of sIgM-inducing factor on the development of sIgM-positive bursocytes sampled at E10 (A), E11 (B), E12 (C) and E13 (D).

Open columns and shaded columns represent sIgM-positive rates of bursocytes incubated with culture medium (controls) and with sIgM-inducing factor for 0 to 96 hours, respectively. * = significantly different from means of controls ($p < 0.05$).

rates in sIgM-inducing factor-treated cells and control cells incubated for 48 and 96 hours ($P < 0.05$). Figure 2-B shows the sIgM-positive rates of E11 bursacytes. The rate was significantly increased by incubation with the factor for 24, 48 and 96 hours ($p < 0.05$). Figure 3-C and figure 3-D also show the positive rates of E12 cells were significantly increased by incubation with the factor for 96 hours ($p < 0.05$) and those of E13 cells by incubation for 24 and 96 hours ($p < 0.05$).

The increase indices (sIgM-positive rates in cells incubated with sIgM-inducing factor/those in cells incubated with culture medium) are shown in Fig. 3. The indices tended to be higher in E11 cells incubated for 24 and 48 hours than those of cells obtained on other days of embryogenesis. After 96 hours of incubation, the increase index of E13 bursacytes incubated with the factor tended to be higher than those of bursacytes obtained on other days of embryogenesis. These results suggest that sensitivity to the sIgM-inducing factor varies with the differentiation period of embryonic bursal lymphoid cells, and that the sensitivity was highest at E11 in the chick embryo examined in this study.

Discussion

In chick embryos, it has been reported that lymphoid stem cell enter the bursa from E7 to E14, and differentiate in the microenvironment presented by reticuloepithelial cells in the lymphoid follicles of the bursa⁷. Differentiation of lymphoid cells can be monitored by measuring the expression of various molecules located on the cell surface. Among these molecules, sIgM is a molecule which functions as an antigen receptor of B lymphocytes^{9,13,15}, and is expressed on B lymphocytes after the rearrangement of antibody genes. Thus, sIgM

expression is thought to be a critical phase of B cell development in the bursa.

According to previous reports concerning to B cell development of the bursa of chicken^{6,8,12}, sIgM-bearing cells in the bursa of chick embryos are detected for the first time on the 12–13th day of embryogenesis. However, in the present experiment, sIgM-positive cells were detected in the bursa at an earlier stage of embryogenesis, although the low positive rate observed at E10 may be due to unidentified artifacts. Reason for the difference in our results and previous results is unclear. However, differences in the titers and specificities of antibodies used in these experiments are possible reason for the discrepancy in the results.

Responsiveness to sIgM-inducing factor (sIgM-positive rate in the cells incubated with sIgM-inducing factor that in cells incubated with culture medium) was low in bursacytes sampled at E10, as well as sIgM-positive rate in bursacytes was low at E10. Responsiveness to sIgM-inducing factor was elevated at E11. The responsiveness at E11 was higher than that of bursacytes sampled at E12 and E13 (except for that of bursacytes sampled at 13E and incubated for 96 hours). These results suggest that responsiveness to sIgM-inducing factor varies with the development of the chick embryo during the B precursor cell-receptive period of the bursa. The 11th day of embryogenesis is suggested to be the beginning of a critical stage of B-lymphocytegenesis in the bursa of chick embryos because the sIgM-positive ratio and responsiveness to sIgM-inducing factor are strongly elevated in this period. On the other hand, reason for the marked increase index of E13 bursacytes after 96 hours of incubation is not clear now. There may be another critical point of B lymphocyte development at a later stage of the B precursor-receptive period.

Surface IgM-inducing factor is thought to act on B lymphoid cells through a receptor assumed to be present on B cell membrane because of its peptide-like nature⁵, although the mechanism of action of the factor is unclear. Regarding quantitative and qualitative variations during B cell differentiation in the bursa, it is suspected that the density and affinity of the receptor that bind to sIgM-inducing factor vary with the development of chick embryos. On the other hand, it is possible that changes in the characteristics of not only lymphoid cells but also bursal epithelial cells occur during the development of chick embryos. The level of production of factors involved in B cell differentiation may also vary with the development of chick embryos. Further studies will be required to elucidate the role of sIgM-inducing factor in B cell development in the bursa of chickens.

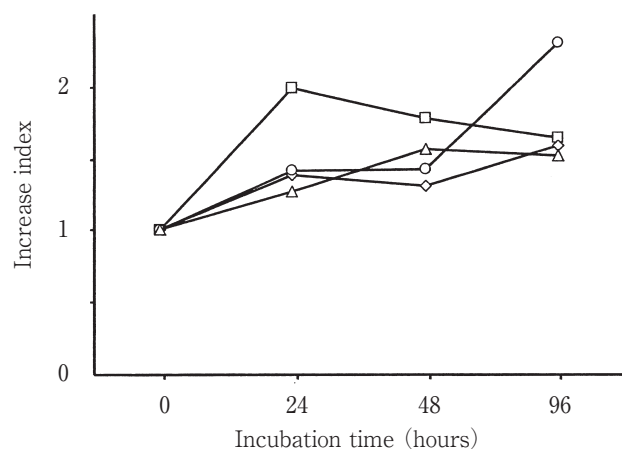


Fig. 3 Increase index of sIgM-inducing factor (sIgM-positive rate in cells incubated with sIgM-inducing factor/ that in cells incubated with culture medium) in bursacytes obtained at E10 (◇), E11 (□), E12 (△) and E13 (○).

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ニワトリ胚のファブリシウス嚢におけるBリンパ球の 発達に対する sIgM 誘導因子の役割

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ファブリシウス嚢はBリンパ球分化と増殖のための中枢リンパ組織として鳥類の免疫機能の発達において重要な役割を演じている。培養ファブリシウス嚢上皮細胞は胚のファブリシウス嚢リンパ球に膜のIgM分子(sIgM)の発現を誘導する因子を産生することが報告されている。ファブリシウス嚢リンパ球のsIgM発現誘導因子に対する感受性はファブリシウス嚢における分化の時期において変動する可能性が考えられる。ニワトリの胚では、リンパ球系の前駆細胞は胚発達の7日目から14日目の間においてのみファブリシウス嚢に進入・定着することが知られている。そこで本研究では、リンパ球系前駆細胞がファブリシウス嚢に定着するこの時期(10日胚から13日胚)におけるファブリシウス嚢リンパ球のsIgM発現の変動を調べるとともに、これらの細胞におけるsIgM発現誘導因子に対する感受性の変動について測定した。sIgM陽性細胞の割合は11日胚で有意に上昇した。sIgM発現誘導因子に対する反応性はこの時期の胚発達に伴って変動し、11日胚で高い傾向を示した。これらの結果から、11日胚齢はニワトリ胚のファブリシウス嚢におけるBリンパ球の発達に関して重要な時期であることが示唆された。

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