Effects of age and gonadal steroids on the localization of antigen-presenting cells, and T and B cells in the chicken oviduct

W. M. Zheng¹, Y. Yoshimura^{1*} and T. Tamura²

¹Graduate School for International Development and Cooperation and ²Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima, 739-8528, Japan

The effects of age and gonadal steroids on the localization of immunocompetent cells, including antigen-presenting cells that contain the major histocompatibility complex (MHC) class II antigen, and T and B cells in the chicken oviduct were studied. Oviductal tissues were collected from laying and immature hens treated with diethylstilboestrol (an analogue of oestrogen) or progesterone. Cryostat sections of the tissues were immunostained for MHC class II, CD3 (T-cell antigen) and Bu-1 (immature B-cell antigen), and examined under a light microscope and an image analysis system. MHC class II⁺, CD3⁺ and Bu-1⁺ cells were observed in the mucosal epithelium and stromal connective tissue of both the laying and immature hens. MHC class II⁺ cells in the oviductal stroma appeared in association with oviductal development during sexual maturation and increased with ageing thereafter. The infiltration of CD3⁺ and Bu-1⁺ cells into the oviductal tissues increased in young laying hens compared with immature hens and decreased in old laying hens compared with young laying hens. Diethylstilboestrol increased the population of MHC class II⁺ and CD3⁺ cells in the stroma of the infundibulum and vagina, but had no significant effect on the population of Bu-1⁺ cells in the oviduct of immature hens. Progesterone increased the population of CD3⁺ cells in the stromal tissue of oviductal segments from all hens, and of Bu-1⁺ cells in the mucosal epithelium of the infundibulum and magnum, but had little effect on the frequency of MHC class II⁺ cells in the oviduct of immature hens. There were typically more immunocompetent cells in the infundibulum and vagina than in the other oviductal segments in laying hens and immature hens treated with sex steroids. These results suggest that local immunity in the chicken oviduct is enhanced during sexual maturation and possibly decreases during ageing. Gonadal steroids may play a significant role in the regulation of local immunity in the oviduct. The effects of oestrogen and progesterone on the influx of these immunocompetent cells into the oviduct differs among cell types and oviductal segments.

Introduction

The chicken oviduct consists of the infundibulum, magnum, isthmus, shell gland and vagina. Egg albumin, shell membrane and shell are formed during the passage of yolk through these oviductal segments. After copulation or artificial insemination, spermatozoa enter the sperm storage tubules in the utero–vaginal junction and participate in fertilization that occurs in the infundibulum (Fujii and Tamura, 1963). Frequently repeated insemination results in a decrease in fertility (Burke and Rieser, 1972; Burke and Yu, 1979; Bakst *et al.*, 1994). The number of leucocytes in the vaginal lumen increases within a few hours of copulation (Higaki *et al.*, 1995). These results suggest that anti-sperm immunoreactions are one of the factors that affect the

survival of spermatozoa in the oviduct. The contamination rate of eggs with pathogenic agents in the oviduct is fairly low even though the vagina opens to the cloaca (Shivaprasad *et al.*, 1990; Poppe *et al.*, 1992). This suggests that entry of foreign pathogens is blocked in the oviduct. Therefore, it is likely that local immune functions in the oviduct exert significant effects on fertility and play an important role in protecting the bird from infection.

The presence of plasma cells and Ig-containing cells in the oviduct of laying hens which may have a role in local immunity has been reported (Van Krey *et al.*, 1987; Kirk *et al.*, 1989; Kimijima *et al.*, 1990; Yoshimura *et al.*, 1997a; Zheng *et al.*, 1997). Lymphoid tissues (Biswal, 1954) and lymphocyte accumulations (Kimijima *et al.*, 1990) in the chicken oviduct have also been observed. Khan *et al.* (1996a,b) reported that both age and the different oviductal segments affect the postnatal development of T-cell subpopulations in the oviduct, and diethylstilboestrol (DES) or DES plus

*Correspondence.

Revised manuscript received 15 April 1998.

progesterone stimulate the infiltration of T cells into the oviduct of immature hens. Injection of immature hens with DES results in an increase in Ig-containing cells in the oviductal epithelium and stroma, but injection of progesterone does not have the same effect (Zheng *et al.*, 1997). In chickens, MHC class II molecules are expressed in antigen presenting cells (Veromaa *et al.*, 1988a; Alberts *et al.*, 1994), and CD3 and Bu-1 antigens are expressed on the cell membrane of functional T lymphocytes (Alberts *et al.*, 1994; Chen *et al.*, 1994) and immature B lymphocytes (Veromaa *et al.*, 1988b; Houssaint *et al.*, 1989). Previous studies have indicated that there are more immunocompetent cells, including MHC class II⁺, CD3⁺ and Bu-1⁺ cells, in the mucosal surface of laying hens than in moulting hens (Yoshimura *et al.*, 1997a). However, the age-related changes in the localization of major immunocompetent cells in the oviduct and their control mechanism are unknown. The aim of the present study was to determine the effects of age and gonadal steroids on the localization of MHC class II⁺, CD3⁺ and Bu-1⁺ cells in the chicken oviduct.

Materials and Methods

Treatment of birds

White Leghorn chickens were kept in individual cages under a light regimen of 14 h light:10 h dark, and were provided with food and water *ad libitum*. Three groups of hens ($n = 5$ in each group) of different ages were used: 60-day-old immature hens, 175-day-old virgin young laying hens and 620-day-old virgin old laying hens; laying hens were laying five eggs or more in a sequence. The laying hens were used 6 h after oviposition, that is, when there was an egg in the shell gland. The effects of gonadal steroids were observed by injecting immature hens (84-days-old) i.m. on a daily basis with 1 mg DES (Nacalai Tesque Inc., Kyoto) or 1 mg progesterone (Sigma Co., St Louis, MO) for 7 days. The steroids were dissolved in sesame oil (vehicle) at a concentration of 10 mg ml⁻¹. Controls were injected with 0.1 ml vehicle. Five birds were used in each group.

Birds were killed by decapitation. The oviduct was removed and small pieces of tissue (approximately 8 mm long) were cut from the middle part of the infundibulum, magnum, isthmus, shell gland and vagina. The tissues were immediately embedded in OCT compound (Miles Inc., Elkhart, IN), snap-frozen in a mixture of absolute isopentane and small pieces of solid carbon dioxide and stored at -20°C until use.

Immunocytochemistry

Antibodies. The primary antibodies used in this study were mouse anti-chicken MHC class II (Veromaa *et al.*, 1988a), mouse anti-chicken CD3 (Southern Biotech Associates Inc., Birmingham, AL), and mouse anti-chicken Bu-1 that recognizes immature B cells but not plasma cells (Veromaa *et al.*, 1988b). Primary antibodies were diluted at a concentration of 1:500 with PBS containing 0.05% (w/v) BSA.

The S-HRP staining kit (for detection of mouse IgG, IgA and IgM; Seikagaku Kogyo Co., Tokyo) was used to reveal the immunoreaction products of the primary antibodies.

Immunostaining. Cryostat sections (15 µm thick) of the oviductal tissues were air-dried on microscope slides pretreated with 2% (v/v) 3-aminopropyl triethoxysilane in absolute acetone. Sections were fixed with absolute acetone at 4°C for 10 min, and air-dried again. They were then rinsed in PBS for 5 min, treated with absolute methanol for 15 min, and washed with PBS for 15 min (3 × 5 min). Sections were first incubated with 10% normal goat serum for 15 min, followed by incubation with primary antibody for 2 h. After washing with PBS (3 × 5 min), the S-HRP staining kit was applied according to the manufacturer's instructions. Briefly, the sections were incubated with biotinylated anti-mouse Igs (IgG, IgM and IgA) for 1 h, with avidin-peroxidase complex for 1 h, and then with 0.02% (w/v) 3, 3'-diaminobenzidine tetrahydrochloride and 0.005% (v/v) H₂O₂ in 0.05 mol Tris-HCl l⁻¹ buffer (pH 7.6) to visualize the immunoreaction products. The slides were counterstained with haematoxylin, dehydrated and mounted. Control slides were prepared as described above, except that the primary antibodies were replaced with normal mouse IgG. The control slides showed no positive staining. All procedures were carried out at room temperature.

Observations and analysis of data

Sections were examined under a light microscope. The population of positive cells was analysed using an image analysis computer system (MacAspect, Mitani Co., Fukui) as described by Zheng *et al.* (1997). Briefly, two different areas (approximately 5000–15 000 µm²) in the epithelium, subepithelial stroma (within 50 µm of the boundary of the mucosal epithelium) and the middle part of stroma were examined in one oviductal segment of a bird. The average of the two counts was calculated and expressed as the number of cells in 1 × 10⁴ µm² in the epithelium, subepithelium and the middle part of the stroma of one section. The significance of difference in numbers of cells among treatments and oviductal segments was examined by one way analysis of variance (ANOVA) followed by Duncan's multiple-range test. $P < 0.05$ was considered to be significant.

Results

Age-related changes in the population of immunocompetent cells in the oviduct

The oviductal mucosa consists of the lamina propria and the mucosal epithelium. The mucosal folds were well developed in young and old laying hens but not in immature hens. The tubular glands were developed in the stroma of the magnum, isthmus and shell gland in young and old laying hens. MHC class II⁺ cells were observed in the mucosal tissues of both immature and laying hens (Fig. 1a,b). No significant difference was observed in MHC class II⁺ cell populations in the mucosal epithelium of each oviductal

Fig. 1. Sections of the oviductal vagina of immature and young laying hens immunostained for major histocompatibility complex (MHC) class II, CD3 (T-cell antigen) and Bu-1 (immature B-cell antigen). (a) The vagina of an immature hen immunostained for MHC class II. There are a few positively stained cells, mainly in the subepithelial stroma. (b) The vagina of a young laying hen stained for MHC class II. A few positively stained cells are present in the stroma. (c) The vagina of an immature hen stained for CD3. There are a few positively stained cells in the stroma. (d) The vagina of a young laying hen stained for CD3. There are many positively stained cells in both the mucosal epithelium and the stroma. (e) The vagina of an immature hen stained for Bu-1. Some positively stained cells are present in the stroma. (f) The vagina of a young laying hen stained for Bu-1. Some positively stained cells are present, mainly in the mucosal epithelium. Arrows indicate examples of positively stained cells. E, mucosal epithelium; L, lumen of oviduct; S, stroma. Scale bars represent 20 μ m.

segment among the immature, young and old laying hens (Fig. 2). Within the young laying hens, there was a significantly greater population of MHC class II⁺ cells in the mucosal epithelium of the isthmus and vagina than in that of the infundibulum. The frequencies of MHC class II⁺ cells in the subepithelial stroma of the infundibulum and magnum were significantly higher in young laying hens than in

immature hens. The population of MHC class II⁺ cells in the subepithelial stroma of each oviductal segment of old laying hens was significantly higher than in young laying hens. Within the immature hens, there was a significantly higher population of MHC class II⁺ cells in the subepithelial stroma of the vagina than in the infundibulum, magnum and shell gland. In old laying hens, the frequency of MHC class II⁺ cells

Fig. 2. Frequency of cells positive for major histocompatibility complex (MHC) class II in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct of (□) immature, (▨) young and (■) old laying hens. Each bar represents the mean $\pm$ SEM of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). In each oviductal segment, bars with different superscripts ^{mn} are significantly different ($P < 0.05$). Within the same treatment, bars with different grouped superscripts ^{abcd} or ^{uv} are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

in the subepithelial stroma was significantly higher in the infundibulum than in the other segments. In addition, there were significantly greater numbers of MHC class II⁺ cells in the subepithelial stroma of the vagina than in the isthmus and shell gland, and greater numbers in the magnum compared with the isthmus. In the middle part of the stroma, the differences in the populations of MHC class II⁺ cells were not significant between the immature hens and the young laying hens, whereas the frequency in the old laying hens in each oviductal segment was significantly higher than the young laying hens. Within the immature and young laying hens, there were no significant differences in MHC class II⁺

cell populations among oviductal segments in the middle part of the stroma. In old laying hens, the infundibulum had a significantly higher population of MHC class II⁺ cells in the middle stroma than the magnum, isthmus and vagina.

CD3⁺ cells were localized in the mucosal epithelium and stroma of the oviduct in immature and laying hens (Fig. 1c, d). The population of CD3⁺ cells in the mucosal epithelium of all oviductal segments was significantly higher in young laying hens than in immature hens, but in old laying hens it decreased significantly in the isthmus and shell gland compared with the young laying hens (Fig. 3). In the mucosal epithelium of old laying hens, the vagina had a significantly higher population of CD3⁺ cells than the magnum, isthmus and shell gland, and the infundibulum had a significantly higher population than the shell gland. In the subepithelial stroma, the population of CD3⁺ cells was significantly higher in young laying hens than in immature hens in all segments, and it was also significantly lower in old laying hens than in young laying hens in the magnum, isthmus and shell gland. Within the old laying hens, there was a significantly higher CD3⁺ cell population in the subepithelial stroma of the vagina than in that of the magnum, isthmus and shell gland. In the middle part of the oviductal stroma, the frequency of CD3⁺ cells in each oviductal segment of the young laying hens was significantly higher than in that of immature hens. However, the frequency of CD3⁺ cells was significantly lower in all oviductal segments of old laying hens than of young laying hens with the exception of the infundibulum. In young laying hens, the frequency of CD3⁺ cells in the vagina was significantly higher than in the isthmus and shell gland, and in old laying hens the frequency in the infundibulum and vagina was significantly higher than in the magnum.

Bu-1⁺ cells were observed in the oviductal mucosal epithelium and stromal connective tissues in immature and laying hens (Fig. 1e, f). The frequency of Bu-1⁺ cells was significantly higher in young laying hens than in immature hens in the mucosal epithelium of each oviductal segment except for the magnum, and it significantly decreased in old laying hens compared with young laying hens in the infundibulum, isthmus and shell gland (Fig. 4). In young laying hens, the frequency of Bu-1⁺ cells in the epithelium of the infundibulum was significantly higher than in that of the magnum, and in the old laying hens the frequency in the vagina was significantly higher than in the isthmus and shell gland. In the subepithelial stroma, the frequency of Bu-1⁺ cells was significantly greater in the infundibulum and isthmus of young laying hens than in immature hens. No significant difference was observed in the frequency of Bu-1⁺ cells in the subepithelial stroma of young and old laying hens. In young laying birds, the frequency of Bu-1⁺ cells in the subepithelial stroma of the infundibulum was significantly greater than in that of the magnum and shell gland, and in the old laying hens the frequency in the vagina was significantly higher than in the magnum and shell gland. In the middle part of the stroma, no significant difference was observed in the Bu-1⁺ cell population among the immature, young and old laying hens or among oviductal segments within each age group.

Fig. 3. Frequency of cells positive for CD3 (T-cell antigen) in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct of (□) immature, (▨) young and (■) old laying hens. Each bar represents the mean of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). In each oviductal segment, bars with different superscripts ^{mno} are significantly different ($P < 0.05$). Within the same treatment, bars with different grouped superscripts ^{abc} or ^{uv} are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

Effects of gonadal steroids on the localization of oviductal immunocompetent cells in immature hens

The oviducts of immature hens treated with DES were more developed in appearance and were of significantly greater mass than those of progesterone-treated and control hens (mean $\pm$ SEM: 6.96 ± 0.05 g in DES-treated birds, 0.32 ± 0.04 g in progesterone-treated birds and 0.28 ± 0.02 g in control birds; $n = 5$ birds in each group). In the oviducts of DES-treated immature hens, the mucosal tissue was well developed including secondary folds in all oviductal segments and tubular glands in the magnum, isthmus and shell gland. There were fewer mucosal folds and no tubular

Fig. 4. Frequency of cells positive for Bu-1 (immature B-cell antigen) in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct of (□) immature, (▨) young and (■) old laying hens. Each bar represents the mean $\pm$ SEM of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). In each oviductal segment, bars with different superscripts ^{mno} are significantly different ($P < 0.05$). Within the same treatment, bars with different grouped superscripts ^{ab} or ^{uv} are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

glands in all oviductal segments of the progesterone-treated and control hens.

MHC class II⁺ cells were observed in the mucosal epithelium and the stroma in all oviductal segments of immature hens treated with gonadal steroids, whereas the subepithelial stroma contained more positive cells than the mucosal epithelium and the middle part of the stroma (Fig. 5a, b). DES and progesterone had no significant effects on the frequencies of MHC class II⁺ cells in the mucosal epithelium within each segment compared with the control hens (Fig. 6). In addition, no significant difference was observed among the oviductal segments within each treatment. DES significantly increased the frequencies of

Fig. 5. Sections of the vagina of immature hens treated with diethylstilboestrol (DES) or progesterone immunostained for major histocompatibility complex (MHC) class II, CD3 (T-cell antigen) and Bu-1 (immature B-cell antigen). (a) The vagina of an immature hen treated with DES stained for MHC class II. There are many positively stained cells mainly in the subepithelial stroma. (b) The vagina of an immature hen treated with progesterone stained for MHC class II. There are a few positively stained cells in the subepithelial stroma. (c) The vagina of an immature hen treated with DES stained for CD3. There are many positively stained cells in the mucosal epithelium and stroma. (d) The vagina of an immature hen treated with progesterone stained for CD3. There are many positively stained cells in the mucosal epithelium and subepithelial stroma. (e) The vagina of an immature hen treated with DES stained for Bu-1. There are positively stained cells in both the mucosal epithelium and the stroma. (f) The vagina of an immature hen treated with progesterone stained for Bu-1. A few positively stained cells are present in the subepithelial stroma. Arrows and arrowheads indicate examples of positively stained cells in the stroma and mucosal epithelium, respectively. E, mucosal epithelium; L, lumen of the oviduct; S, stroma. Scale bars represent 20 μ m.

MHC class II⁺ cells in the subepithelial stroma of the infundibulum and vagina compared with the control, whereas progesterone did not have such an effect. In the DES-treated immature hens, the population of MHC class II⁺

cells in the subepithelial stroma of the infundibulum was significantly greater than in that of the magnum, isthmus and shell gland. DES and progesterone did not affect the frequency of MHC class II⁺ in cells the middle part of the

Fig. 6. Frequency of cells positive for major histocompatibility complex (MHC) class II in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct in immature hens treated with (▨) diethylstilboestrol (DES) or (■) progesterone; (□) control. Each bar represents the mean $\pm$ SEM of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). Within each oviductal segment, bars with different superscripts ^{mn} are significantly different ($P < 0.05$). In the subepithelial stroma of DES-treated immature hens, bars with different superscripts ^{ab} are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

stroma with the exception that progesterone increased the frequency in the shell gland. No other significant differences among treatments or oviductal segments were observed in the middle part of the stroma.

CD3⁺ cells were observed in the mucosal epithelium and stromal connective tissue of all oviductal segments in immature hens treated with DES and progesterone (Fig. 5c, d). The frequency of CD3⁺ cells in the mucosal epithelium of the vagina was significantly increased by progesterone compared with the control group, whereas no significant increase was observed in DES-treated birds (Fig. 7). Within the progesterone-treated immature hens, the frequency of CD3⁺ cells in the mucosal epithelium of the vagina was significantly greater than in the other oviductal segments. Progesterone significantly increased the frequencies of CD3⁺ cells in the subepithelial stroma of all oviductal segments with the exception of the infundibulum.

Fig. 7. Frequency of cells positive for CD3 (T-cell antigen) in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct in immature hens treated with (▨) diethylstilboestrol (DES) or (■) progesterone; (□) control. Each bar represents the mean $\pm$ SEM of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). Within each oviductal segment, bars with different superscripts ^{mn} are significantly different ($P < 0.05$). Within the same treatment, bars with different superscripts ^{ab} for the control, ^{uvw} for the DES-treated, and ^{xy} for the progesterone-treated immature hens are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

DES caused a significant increase of these cells in the infundibulum and vagina. In the control hens, the frequency of CD3⁺ cells in the subepithelial stroma of the vagina was significantly higher than in the infundibulum, magnum and isthmus. In the DES-treated immature hens, the frequencies of CD3⁺ cells in the subepithelium of the infundibulum and vagina were significantly greater than those in the magnum, isthmus and shell gland. In the middle part of the stroma, the frequencies of CD3⁺ cells were significantly increased in the isthmus, shell gland and vagina by progesterone, and in the infundibulum and vagina by DES. Within the control hens, the frequency of CD3⁺ cells in the middle part of the stroma was significantly greater in the magnum than in the infundibulum. In the DES-treated birds there were significantly greater numbers of CD3⁺ cells in the middle part of the stroma in the infundibulum and vagina than in the magnum and shell gland.

Bu-1⁺ cells were localized in the mucosal epithelium and the stromal connective tissue in all oviductal segments of immature hens treated with DES and progesterone (Fig. 5e, f). Progesterone significantly increased the frequencies of Bu-1⁺ cells in the mucosal epithelium in the infundibulum and magnum, whereas DES had no significant effect (Fig. 8). In the progesterone-treated immature hens, there was a higher frequency of Bu-1⁺ cells in the infundibulum and magnum than in the isthmus and shell gland. Both DES and progesterone had no significant effect on the frequencies of Bu-1⁺ cells in the subepithelial stroma compared with the control birds. In the control hens, the frequency of Bu-1⁺ cells in the subepithelial stroma was greater in the magnum than in the shell gland, and in the progesterone-treated immature hens it was greater in the magnum than in the isthmus, shell gland and vagina. Progesterone significantly increased the Bu-1⁺ cell population in the middle part of the stroma of the infundibulum and DES had the same effect in the vagina. In the control hens, the middle part of the stroma of the magnum contained significantly more Bu-1⁺ cells than the infundibulum and the shell gland. In the progesterone-treated group, the Bu-1⁺ cell population was greater in the magnum than in the isthmus, shell gland and the vagina, and in the DES-treated hens, it was greater in the vagina than in the isthmus.

Discussion

The significant findings of this study are that the frequencies of MHC class II⁺, and of T and B cells in the chicken oviduct increase during sexual maturation, and the population of MHC class II⁺ cells increases further during ageing after sexual maturation, but the populations of T and B cells decrease. In addition, both oestrogen and progesterone affect the influx of immunocompetent cells into the oviduct of immature hens.

The MHC class II antigen is known to be present on antigen-presenting cells such as macrophages in chickens (Ewert *et al.*, 1980), and it is closely associated with the function of helper T cells in immune responses (Nordskog *et al.*, 1987; Vainio *et al.*, 1987; Alberts *et al.*, 1994). In this study, MHC class II⁺ cells were observed in the oviductal mucosal epithelium and stroma of all birds. Young laying hens had a higher population of MHC class II⁺ cells than immature hens in the subepithelial stroma of the infundibulum and magnum. Injection of immature hens with DES stimulated oviductal development and increased the frequencies of MHC class II⁺ cells in the subepithelial stroma of the infundibulum and vagina. Progesterone did not have the same effect. It is possible that during sexual maturation the number of MHC class II⁺ cells increases in association with development of the oviduct under the control of oestrogen. The frequency of MHC class II⁺ cells in the oviductal stroma of old laying hens was significantly higher than in young laying hens. The production of oestrogen decreases in old laying hens compared with young laying hens (Bahr and Palmer, 1989). Therefore, it is assumed that oestrogen is not responsible for the further increase in MHC class II⁺ cells in the oviduct of old laying hens. The increases in the

Fig. 8. Frequency of cells positive for Bu-1 (immature B-cell antigen) in the (a) mucosal epithelium, (b) subepithelial stroma and (c) middle part of the stroma of the oviduct in immature hens treated with (▨) diethylstilbestrol (DES) or (■) progesterone; (□) control. Each bar represents the mean $\pm$ SEM of the number of positive cells in $1 \times 10^4 \mu\text{m}^2$ tissue ($n = 5$ birds in each bar). Within each oviductal segment, bars with different superscripts ^{mn} are significantly different ($P < 0.05$). Within the same treatment, bars with different superscripts (^{ab} for the control, ^{uv} for the DES-treated, and ^{xy} for the progesterone-treated immature hens) are significantly different ($P < 0.05$). In, infundibulum; Ma, magnum; Is, isthmus; Sg, shell gland; Va, vagina.

population of these cells in old laying hens may be caused by long term exposure of the oviduct to foreign agents. Further investigations are needed to determine the factors that are responsible for the increase of MHC class II⁺ cells in the oviduct of old laying hens.

The monoclonal antibody to chicken CD3 recognizes the CD3 antigen on the surface of mature T cells (Chen *et al.*, 1994). In this study, the oviductal CD3⁺ cell population increased with sexual maturation and decreased with ageing. In addition, injection of immature hens with DES or progesterone caused the infiltration of CD3⁺ cells into the mucosal tissues of the oviduct. An age-related decline in T-cell function, including the appearance of a non-functional subset of T cells and loss of T-cell proliferative ability, has been observed in rodents and humans (Song *et al.*, 1993). A decline in T-cell function may also occur during ageing in

hens. A decrease in circulating gonadal steroid concentrations may be partly responsible for the decrease in oviductal T-cell population in old laying hens. Khan *et al.* (1996a) reported that injection of 7-day-old hens with DES or DES plus progesterone, but not progesterone alone, caused depopulation of T lymphocytes from the thymus, spleen and caecal tonsil, and subsequent infiltration into the oviduct. These results differ from the present study in which progesterone stimulated infiltration of T cells into the oviduct. The reason for this difference is unknown. However, it is possible that the 7-day-old hens used by Khan *et al.* (1996a) were less sensitive to progesterone than the 84-day-old hens used in the present study.

The monoclonal antibody to Bu-1 used in this study recognizes immature B cells but not the antibody-producing plasma cells that are mature B cells (Veromaa *et al.*, 1988b; Mansikka *et al.*, 1989; Tregaskes *et al.*, 1996). A significantly higher population of Bu-1⁺ cells was observed in the mucosal epithelium and subepithelial stroma of most oviductal segments in young laying hens compared with immature hens. In contrast, no significant difference was observed in the frequencies of Bu-1⁺ in the middle part of the stroma in each oviductal segment between young laying hens and immature hens. Injection of immature hens with progesterone increased the Bu-1⁺ cell frequencies in the infundibulum and magnum, mainly in the mucosal epithelium. In contrast, DES had no significant effect on the localization of Bu-1⁺ cells in the oviduct with the exception of the middle part of the stroma in the vagina. These results suggest that in chickens oviduct Bu-1⁺ cells are located mainly in the mucosal surface tissues in laying hens and in immature hens treated with sex steroids. These results support previous findings (Yoshimura *et al.*, 1997a). In old laying hens, as compared with young laying hens, the frequency of Bu-1⁺ cells decreased in the mucosal epithelium in the infundibulum and isthmus, whereas there was no significant change in frequency in the middle part of the stroma. It is likely that during ageing the reduction in the Bu-1⁺ cell population occurs mainly in the mucosal epithelium. Receptors for oestrogen and progesterone have been identified in the bursa of Fabricius tissue (Sullivan and Wira, 1979). Severe atrophy of the bursa and an increase in plasma cell population in the oviduct have been observed in DES-treated chickens (Khan *et al.*, 1997). It has also been reported that oestrogen increases the plasma concentration of Ig in broiler chicks (Leitner *et al.*, 1996), and causes an increase in Ig-containing cells in the oviduct, whereas progesterone does not show such effects (Zheng *et al.*, 1997). Studies in mice showed that oestrogen enhanced B-cell maturation and increased antibody production (Wira and Sandoe, 1980; Paavonen *et al.*, 1981). Therefore, the age-related changes in Bu-1⁺ cell frequency in the oviduct may be affected by gonadal steroids. Progesterone possibly increases Bu-1⁺ cells in the oviduct, whereas oestrogen may increase the population of plasma cells rather than Bu-1⁺ cells.

Immunocompetent cells may have a role in the local immunity of the oviduct. In this study there were typically more immunocompetent cells in the infundibulum and vagina than in the other oviductal segments in laying hens and immature hens treated with sex steroids. Similar

findings were also reported by Withanage *et al.* (1997) and Yoshimura *et al.* (1997a). Zheng *et al.* (1997) reported that the population of Ig-containing cells in laying hens and immature hens treated with DES was higher at the two ends of the oviduct. It may be concluded that specific immune functions (humoral immunity and cell-mediated reactions) in the chicken oviduct are stronger in the infundibulum and vagina in both sexually matured hens and immature hens treated with sex steroids. This may contribute to protection of the oviduct from infection as the vagina opens to the cloaca that contains numerous exogenous pathogens (Shivaprasad *et al.*, 1990; Poppe *et al.*, 1992). This may also influence the storage, selection and survival of spermatozoa as the number of leucocytes in the vaginal lumen increases within a few hours after copulation and some are in close contact with spermatozoa (Higaki *et al.*, 1995; Yoshimura *et al.*, 1997b). In the infundibulum, spermatozoa that did not participate in fertilization were phagocytosed by macrophages, suggesting that an anti-sperm immunoreponse occurs (Koyanagi and Nishiyama, 1981).

In conclusion, the present results suggest that both cellular and humoral immunity in the oviduct increase during sexual maturation. During ageing, T- and B-cell populations decrease, whereas antigen-presenting cells containing MHC class II antigen increase. These age-related changes in the frequency of immunocompetent cells in the oviduct may be correlated with the secretion of gonadal steroids. It is likely that the effects of oestrogen and progesterone on the influx of immunocompetent cells differ among the cell types and oviductal segments. The local immune function is likely to be more active in the infundibulum and vagina, which would contribute to protection of the oviduct from infection and would also affect the fertilizing ability of spermatozoa.

The authors thank O. Vainio, Department of Medical Microbiology, University of Turku, Finland, for his kind donation of antibodies to chicken MHC class II and Bu-1.

References

- Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson D (1994) The immune system. In *Molecular Biology of the Cell* 3rd Edn pp 1228–1251 Eds M Robertson and R Adams. Garland Publishing Inc., New York
- Bahr JM and Palmer SS (1989) The influence of aging on ovarian function *Critical Reviews in Poultry Biology* 2 103–110
- Bakst MR, Wishart G and Brillard JP (1994) Oviductal sperm selection, transport and storage in poultry *Poultry Science Review* 5 117–143
- Biswal G (1954) Additional histological findings in the chicken reproductive tract *Poultry Science* 33 843–851
- Burke WH and Rieser JW (1972) The effects of anti-sperm antisera and uterine fluids on the fertilizing ability of turkey spermatozoa *Poultry Science* 51 190–195
- Burke WH and Yu WCY (1979) Infertility in the turkey. I. Effects of anti-sperm immune globulins on fertilizing ability of turkey spermatozoa *Poultry Science* 58 1367–1371
- Chen CH, Gobel TWE, Kubota T and Cooper MD (1994) T-cell development in the chicken *Poultry Science* 73 1012–1018
- Ewert DL, Gilmour DG, Briles WE and Cooper MD (1980) Genetics of Ia-like alloantigens in chickens and linkage with B major histocompatibility complex *Immunogenetics* 10 169–174
- Fujii S and Tamura T (1963) Location of sperm in the oviduct of the domestic fowl with special reference to storage of sperm in the vaginal gland *Journal of the Faculty of Fisheries and Animal Husbandry, Hiroshima University* 5 145–163

- Higaki K, Yoshimura Y, Tamura T and Okamoto T (1995) Localization of spermatozoa and leukocytes in vagina and utero-vaginal junction after copulation in Japanese quail (*Coturnix coturnix japonica*) *Japanese Poultry Science* **32** 387–393
- Houssaint E, Lassila O and Vainio O (1989) Bu-1 antigen expression as a marker for B-cell precursors in chicken embryos *European Journal of Immunology* **19** 239–243
- Khan MZI, Hashimoto Y, Iwami Y and Iwanaga T (1996a) Hormonal regulation of T-cell subsets in the oviduct: an immunohistochemical study using sex-hormone-treated chickens *Journal of Veterinary Medicine Science* **58** 1161–1167
- Khan MZI, Hashimoto Y, Konno A, Kon Y and Iwanaga T (1996b) Development of T-lymphocyte subpopulations in the postnatal chicken oviduct *Cell Tissue Research* **284** 317–325
- Khan MZI, Hashimoto Y, Iwami Y and Iwanaga T (1997) Postnatal development of B lymphocytes and immunoglobulin-containing plasma cells in the chicken oviduct: studies on the cellular distribution and influence of sex hormones *Veterinary Immunology and Immunopathology* **56** 329–338
- Kimijima T, Hashimoto Y, Kitagawa H, Kon Y and Sugimura M (1990) Localization of immunoglobulins in chicken oviduct *Japanese Journal of Veterinary Science* **52** 299–305
- Kirk TA, Van Krey HP, Hulet RM, Dunnington EA and Denbow DM (1989) The relationship of infertility to antibody production in the uterovaginal sperm storage tubules of turkey breeder hens *Theriogenology* **31** 955–961
- Koyanagi F and Nishiyama H (1981) Fate of spermatozoa that do not participate in fertilization in the domestic fowl *Cell Tissue Research* **214** 89–95
- Leitner G, Landsman T, Blum O, Zaltsmann N and Heller ED (1996) Effects of gonadal steroids and their antagonists on the humoral immune response of immune-selected broiler chicks *Poultry Science* **75** 1373–1382
- Mansikka A, Veromaa T, Vainio O and Toivanen P (1989) B-cell differentiation in the chicken: expression of immunoglobulin genes in the bursal and peripheral lymphocytes *Scandinavian Journal of Immunology* **29** 325–331
- Nordskog AW, Pevzner IY and Lamont SJ (1987) Subregions and functions of the chicken major histocompatibility complex *Poultry Science* **66** 790–794
- Paavonen T, Anderson LC and Adlercreutz H (1981) Sex hormone regulation of *in vitro* immune response: estradiol enhances human B-cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures *Journal of Experimental Medicine* **154** 1935–1945
- Poppe C, John RP, Forsberg CM and Irwin RJ (1992) *Salmonella enteritidis* and other *Salmonella* in laying hens and eggs from flocks with *Salmonella* in their environment *Canadian Journal of Veterinary Research* **56** 226–232
- Shivaprasad HL, Timoney JE, Mordes S, Lucio B and Baker RC (1990) Pathogenesis of *Salmonella enteritidis* infection in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses *Avian Diseases* **34** 548–557
- Song L, Kim YH, Chopra RK, Proust JJ, Nagel JE, Nordin AA and Adler WH (1993) Age-related effects of T cell activation and proliferation *Experimental Gerontology* **28** 313–321
- Sullivan DA and Wira CR (1979) Sex hormone and glucocorticoid receptors in the bursa of Fabricius of immature chickens *Journal of Immunology* **122** 2617–2623
- Tregaskes CA, Bumstead N, Davison TF and Young JR (1996) Chicken B-cell marker chB6 (Bu-1) is a highly glycosylated protein of unique structure *Immunogenetics* **44** 212–217
- Vainio O, Toivanen P and Toivanen A (1987) Major histocompatibility complex and cell cooperation *Poultry Science* **66** 795–801
- Van Krey HP, Schuppin GT, Denbow DM and Hulet RM (1987) Turkey breeder hen infertility associated with plasma cells in the uterovaginal sperm storage glands *Theriogenology* **27** 913–921
- Veromaa T, Vainio O, Jalkanen S, Eerola E, Cranfors K and Toivanen P (1988a) Expression of B-L and Bu-1 antigens in chickens bursectomized at 60 h of incubation *European Journal of Immunology* **18** 225–230
- Veromaa T, Vainio O, Eerola E and Toivanen P (1988b) Monoclonal antibodies against chicken Bu-1a and Bu-1b alloantigens *Hybridoma* **7** 41–48
- Wira CR and Sandoe CP (1980) Hormonal regulation of immunoglobulins: influence of estradiol on immunoglobulins A and G in the rat uterus *Endocrinology* **106** 1020–1026
- Withanage GSK, Baba E, Sasai K, Fukata T, Kuwamura M, Miyamoto T and Arakawa A (1997) Localization and enumeration of T and B lymphocytes in the reproductive tract of laying hens *Poultry Science* **76** 671–676
- Yoshimura Y, Okamoto T and Tamura T (1997a) Localization of MHC class II, lymphocytes and immunoglobulins in the oviduct of laying and moulting hens *British Poultry Science* **38** 590–596
- Yoshimura Y, Higaki K and Tamura T (1997b) Phagocytosis of sperm by the oviductal mucosa in Japanese quail, *Coturnix coturnix japonica*. *Assisted Reproductive Technology/Andrology* **9** 213–222
- Zheng WM, Yoshimura Y and Tamura T (1997) Effects of sexual maturation and gonadal steroids on the localization of IgG-, IgM- and IgA-positive cells in the chicken oviduct *Journal of Reproduction and Fertility* **111** 277–284