学 位 論 文 の 要 旨

論文題目　Studies on the mucosal barrier system in the oviduct of hens
（ニワトリ卵管の粘膜バリアシステムに関する研究）

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Goal of the study

Immune system in the oviduct is responsible to maintain the health of this organ to produce hygienic eggs. The mucosal barrier function plays essential roles in the defence to pathogens. Mucins, tight junctions of epithelium, and leukocyte activity form mucosal barrier to play roles to prevent infection in the mucosal tissues. Mucins may prevent adherence of pathogens to the mucosal surface. Tight junctions form the outer mechanical barrier to protect the mucosa from being invaded with infectious agents. The goal of this study was to determine the mechanism by which mucosal barrier mediated by mucins and tight junction is formed in the mucosal epithelium of the oviduct. Specifically, it was focused on the mechanism by which mucin synthesis for mucosal barrier is stimulated by oviductal growth, gonadal steroid and bacterial component, LPS, in the lower segment of oviduct, namely vagina, uterus or isthmus. Then, the existence of the epithelial barrier formed by tight junction was also examined.

1. Formation of mucosal surface barrier by mucin in the lower oviductal segments and its changes with egg-laying phase and gonadal steroid stimulation in hens.

Mucins produced by mucosal epithelial cells have the ability to form a physical barrier and act as adhesion decoys to invading agents. The aim of this study was to determine the effects of the egg-laying phase and estradiol on the mucin expression that forms a mucosal surface barrier in the lower oviductal segments in hens. White Leghorn laying and

molting hens were used. Molting hens were given either sesame oil (control groups) or estradiol benzoate (EB groups) via i.m. injection (n = 5 per group). The lower segments of oviduct (vagina, uterus, and isthmus) of these birds were collected. Localization and gene expression of mucosal mucin were analyzed by quantitative RT-PCR and immunohistochemistry. Localization of mucin polysaccharide was performed by alcian blue (AB) staining. Sugar residues were localized by lectin (WGA or Jacalin) histochemistry. In the vagina, uterus and isthmus, mucin expression was formed, and immunoreactive mucin5AC and AB-positive mucopolysaccharide were localized in the mucosal epithelium. Their expression and densities were reduced in molting hens compared with laying hens, and up-regulated by EB. Substances positively stained by WGA and Jacalin were identified on the surface of the mucosal epithelium in the lower oviductal segments in laying and molting hens. These results suggest that mucin synthesis in the lower segments of the oviduct is reduced due to decline of circulating estrogen level, although the existence of WGA- and Jacalin-positive sugars may be kept even in the molting phase.

2. Induction of mucin expression by lipopolysaccharide in the lower oviductal segments in hens

The aim of this study was to determine the effect of lipopolysaccharide (LPS), a component of Gram negative bacteria, on the mucin expression in the lower oviductal segments (vagina and uterus) of hens. The mucosal tissues of the vagina and uterus were collected from White Leghorn laying and molting hens, and molting hens with or without intramuscular injection with 1 mg estradiol-benzoate (EB) daily for 7 d. These tissues were cultured in TCM-199 culture medium with or without LPS (10, 100 or 1000 ng/ml) for 1.5 or 3 h. Then, mucin expression was analyzed by quantitative RT-PCR. Cultured tissues were also processed for paraffin sections and stained with alcian blue (AB). Mucin expression in the cultured vagina and uterus tissues of laying and molting hens was up-regulated by LPS in a dose- and time-dependent manner. However, there was no significant response to LPS for induction of mucin in the tissues of EB-group hens. These results suggest that mucin expression responsible for mucosal barrier is stimulated by LPS in the vagina and uterus of both laying and molting hens. Estrogen may suppress the response to LPS for mucin induction.

3. Toll-like receptor signaling for the induction of mucin expression in response to lipopolysaccharide in hen vagina

TLRs are known to recognize microbial molecular patterns, generally by direct interaction with molecules on the pathogen surface. TLR-4 works to recognize lipopolysaccharide (LPS) of Gram-negative bacteria. The aim of this study was to determine the intracellular signaling molecules for mucin induction by LPS, and the effect of molting and estrogen on their expression. Expression of TLR4, its adaptor molecules, and transcriptional factors in the vaginal mucosa of laying and molting hens treated with or without estradiol were examined by RT-PCR. Expression of mucin in the cultured mucosal tissue stimulated by LPS together with inhibitors of transcriptional factors was analyzed by quantitative RT-PCR. Expression of TLR4, its adaptor molecule, namely, myeloid differentiation factor 88 (MyD88) or toll-interleukin 1 receptor domain-containing adaptor-inducing IFN-β (TRIF), and transcriptional factors, namely, cFos, and cJun, were declined in molting hens compared with laying hens, and was upregulated by estradiol. In mucosal tissue of laying hens, mucin expression was upregulated by LPS, whereas it was suppressed by inhibitors of transcriptional factors, namely, ALLN (an inhibitor of IκB proteolysis), BAY-117085 (a NFκB inhibitor), U0126 (a mitogen-activated protein kinase [MAPK] inhibitor), and Transhinone IIA (an activated protein 1 [AP-1] inhibitor). These results suggest that MyD88-dependent pathway in the downstream of TLR4 and transcriptional factor of NFκB and AP-1 participate in the induction of mucin expression by LPS in the vaginal mucosa. Also, these signaling functions may be declined during molting due to the decline of circulating estrogen level.

4. Expression of tight junction molecule "claudins" in the lower oviductal segments and their changes with egg-laying phase and gonadal steroid stimulation in hens.

Tight junction in the mucosal epithelium plays essential roles as a mucosal barrier to prevent the invasion of microbes into the mucosal tissues. The aim of this study was to determine the effects of egg-laying phase and gonadal steroid on the expression of tight junction molecule "claudins" in the lower oviductal segments in hens. White Leghorn laying and molting hens were used. A proportion of the molting hens were injected with sesame oil (control) or estradiol benzoate (EB). The lower segments of oviduct (isthmus, uterus, and vagina) of these birds were collected. Gene expression of *claudin-1, -3, -5*,

lipopolysaccharide-induced *TNFα* factor (*LITAF*), and *IFNγ* was analyzed by quantitative RT-PCR, and localization of claudin-1 was examined by immunohistochemistry. Permeability in the mucosal epithelium was examined by intrauterine injection of fluorescein isothiocyanate (FITC)-dextran. Expression of *claudin-1, -3,* and *-5* genes and density of claudin-1 protein in the lower oviductal segments were significantly higher in laying hens than in molting hens, and their expression was upregulated by EB. Expression of *LITAF* and *IFNγ* genes was higher in molting hens than in laying hens. More FITC-dextran infiltrated into the intercellular space of the uterus mucosal epithelium in molting group hens than in laying and EB group hens. These results suggest that barrier functions of the mucosal epithelium at the lower oviductal segments may be disrupted due to reduction of claudin expression in molting hens.

5. Conclusion

This study has identified that the mucosal barrier system mediated by mucin and tight junction is formed in hen oviduct. This mucosal barrier system in the oviduct is expected to play important roles to protect the oviductal tissue from infection by pathogenic microorganisms. The expressions of mucin and tight junction molecules were declined in molting hens with regression of oviduct and upregulated by estrogen. Thus, the mucosal barrier system formed by mucin and tight junction are probably weakened due to less circulating estrogen level. It was also established by this study that mucin expression was stimulated by LPS of Gram negative bacteria such as *Salmonella* organism through NFkB and AP-1 mediated manner in the oviduct.