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Modulatory roles of proinflammatory cytokines on the expression of cathelicidins in the lower regions of the oviduct of laying hens

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ABSTRACT

The current study aimed to confirm and examine the physiological roles of the proinflammatory cytokines IL1B and IL6 on the immune functions which mediated by cathelicidins (CATHs) in the uterus and vagina of laying hens. Snaps of the mucosal tissues of uterus and vagina were incubated in culture medium or chicken recombinant IL1B and IL6 for 1.5 h or 3 h before extraction of total RNA to be used for examination of IL1B and IL6, their receptors, and cathelicidins by semi-quantitative PCR; and to examine the changes in cathelicidins expressions by real-time PCR. PCR analysis confirmed that *IL1B* and *IL6*, their receptors, and *CATH1-3* were expressed in the mucosal tissues of the uterus and vagina. In uterus tissue, IL1B did not affect the expression of *CATH1* and *−2* at different doses and incubation time, whereas *CATH3* was significantly downregulated by incubation with IL1B for 1.5 h. In the vaginal tissue, the expressed *CATH1*, *−2* and *−3* were significantly upregulated by incubation with IL1B for 1.5 h in a dose-dependent manner. In uterus tissue, *CATH1* expression was down-regulate by IL6 incubation for 1.5 h, but not by 3 h however, *CATH3* expression was significantly increased by incubation with IL6 for 1.5 h, but not for 3 h. In the vaginal tissues, all *CATHs* expression was not affected significantly by incubation with IL6. These current observations suggest that *CATH1*, *−2* and *−3* in the vagina are upregulated by IL1B, and *CATH3* in the uterus is also upregulated by IL6. IL1B and IL6 synthesized in response to infection by the microbes may enhance the defense system in the oviduct mucosal tissues by increasing the synthesis of CATHs.

1. Introduction

The hen oviduct is the organ responsible for receiving the ovulated egg from the ovary and completing the process of egg formation by secreting albumen, eggshell membrane and egg shell. Its lower parts open to the coloaca, where numerous microorganisms may colonize, by vagina, and thus such parts of the oviduct are more susceptible to infections by them. Therefore, the immunodefence system of the oviduct to prevent the infection is crucial to produce hygienic eggs [1].

The innate immunity is the first protective wall against pathogens that may invade healthy tissues, such process is initiated by recognizing the microbe-associated molecular patterns (MAMPs) by specific receptor families such as Toll-like receptors family (TLRs), intracellular nucleotide-binding oligomerization (NOD)-like receptors (NLRs) [2–5]. The interaction of MAMPs and TLRs leads to synthesizing antimicrobial peptides and cytokines such as interleukin 1β (IL1B) and IL6 [6].

Avian β-defensins (AvBDs) and cathelicidins (CATHs) are the antimicrobial peptides were identified in chicken tissues. They have a potential function to kill a broad spectrum of microbes, including Gram-negative and -positive bacteria, fungi and enveloped viruses [7–9]. Till now fourteen AvBDs genes have been recognized in chickens (*AvBD1 – 14*), and among them expression of eleven *AvBDs* was identified in the oviductal tissues [10–13]. Cathelicidins are commonly expressed in different tissues of animals such as goat, sheep, cattle, mice and human [14–16]. In chicken, four CATHs were identified (*CATH1 – 3* and *CATHB*), and three of them were expressed in the oviduct [17–21].

It was revealed that *in vivo* or *in vitro* stimulation of laying hens or its oviductal tissues with MAMPs enhanced the expression of *AvBDs*, *CATHs* and proinflammatory cytokines, *IL1B* and *IL6*, in the vagina [12,22–24]. The physiological roles of such proinflammatory cytokines on the innate immune functions mediated by antimicrobial peptides were reported; It was observed that IL1B upregulated the expression of β-defensins in mammalian keratinocytes, corneal and uterine epithelium [25–29]. In chicken also, it was reported that AvBD12 in the ovarian follicular tissues, was induced by IL1B [30], and the *AvBDs* expression in the vaginal cultured cells model was induced by

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Table 1
Primer sequences for *ILs*, *ILRs*, *CATHs* and *RPS17*.

Target gene	Sequences 5́–3́	Accession No.	References
IL1B	F: GGGCATCAAGGGCTACAA R: CTGTCCAGGCGGTAGAAGA	NM_204524	[24]
IL6	F: AGAAATCCCTCCTCGCCAAT R: AAATAGCGAACGGCCCTCA	NM_204628.1	[24]
IL1R1	F: TTGTTCAGTGCTGAAGAATGTGTTATTT R: ACGAATGTTCTGAACTGGGTGTTC	NM_205485	[47]
IL6R	F: TGAGGATGATCCCTACGGCTATG R: CCGGCATCATCAGCAGTGT	NM_001044675	[37]
CATH1	F: GCTGTGGACTCCTACAACCAAC R: GGAGTCCACGCAGGTGACATC	NM_001001605.3	[48]
CATH2	F: CAAGGAGAATGGGGTCATCAG R: CGTGGCCCCATTTATTCATTCA	NM_001024830.2	[48]
CATH3	F: GCTGTGGACTCCTACAACCAAC R: TGGCTTTGTAGAGGTTGATGC	NM_001311177.1	[48]
RPS17	F: AAGCTGCAGGAGGAGGAGAGG R: GGTTGGACAGGCTGCCGAAGT	NM_204217	[37]

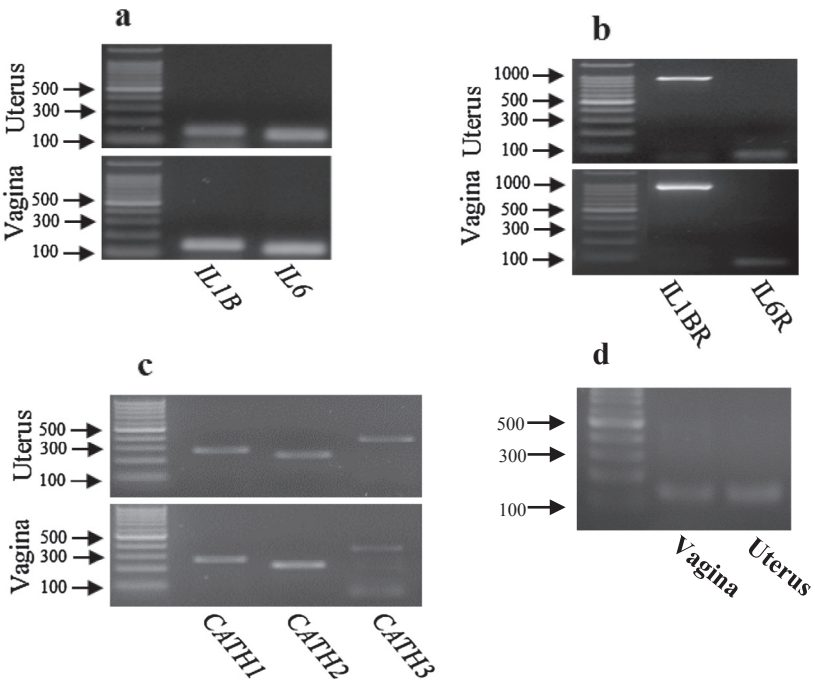

Fig. 1. Reverse transcription-PCR products of the mRNA of the *ILs* (a), *ILRs* (b) *CATHs* (c) and *RPS17* housekeeping gene (d) in the mucosal epithelial tissue of the uterus and vagina of laying hens. Products were separated by electrophoresis on 2% (w/v) agarose gel containing 0.025% (w/v) ethidium bromide.

stimulation with IL1B and IL6 [31]. Also, some studies noticed that the expression of CATHs is regulated by IL6 [32–34]. However, the physiological roles and effects of IL1B and IL6 on the *CATHs* expressed in the lower oviductal parts of hens were not examined. Thus, the current work aims to confirm and examine the physiological roles of IL1B and IL6 on the immune functions of the oviduct of laying hens which mediated by *CATHs* in a tissue cultured model.

2. Materials and methods

2.1. Birds and tissue preparation

White Leghorn laying hens of age 250-d-old which regularly lay four or more eggs in a clutch were used. Each bird was reared in individual cage under lighting regimen consists of 14 h light: 10 h dark. water and feed were provided to them *ad libitum*. They were euthanized with sodium pentobarbital (Somnopentyl; Kyoritsu Pharmaceutical Co., Tokyo, Japan) 6 h after oviposition, namely before the egg enters the uterus. Then the middle parts of the uterine and vaginal mucosal tissues were obtained from five different birds. This study was achieved under the regulation and rules of Hiroshima University for Animal Experimentation.

2.2. Tissue cultures of the uterine and vaginal mucosa

The uterine and vaginal mucosal tissues were collected, then washed with sterilized phosphate-buffered saline including 10 U/ml penicillin and 10 μg/ml streptomycin antibiotics (Cosmo Bio. Co., Ltd., Tokyo, Japan). Small pieces of mucosal tissues (approximately 2 × 2 × 1 mm; 5 pieces/tube) were placed in sterilized tubes for culture (Greiner Bio-One Ltd., Tokyo, Japan) containing 2 ml TCM-199 medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) with 10 U/ml penicillin and 10 μg/ml streptomycin antibiotics. Then chicken recombinant IL1B and IL6 (AbD Serotec., Oxford, UK) were added at concentrations 0 to 10^3 ng/ml for 1.5 h, or at concentrations of 0 to 10^2 ng/ml for 3 h at 39 °C under a 5% CO_2.

2.3. Extraction of RNA

After stimulation, the cultured tissues were collected and prepared for RNA extraction as mentioned in our previous work [22]. Briefly, samples are placed in ice bath and grinded in Sepasol RNA I Super

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