Antimicrobial Peptides Expression for Defense System in Chicken Gastrointestinal and Reproductive Organs

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ABSTRACT: Maintenance of animal health is essential to obtain their maximum productivity and safe products. Avian β-defensins (AvBDs) are the member of antimicrobial peptides, and Toll-like receptors (TLRs) are the primary receptors that recognize pathogen-associated molecular patterns (PAMPs) of microbes. The aim of this study was to characterize the innate immune system with the focus on the expression of AvBDs in the gastrointestinal tract and reproductive organs for the strategy to enhance the disease resistance of chickens. The proventriculus and cecum of broiler chicks expressed *TLRs* and *AvBDs*. It is suggested that a variety of PAMPs of microbes are recognized by different TLRs, probably leading to regulate the synthesis of innate immune factors including AvBDs. In laying hens, *TLRs* and *AvBDs* were expressed in the theca and granulosa layers of ovarian follicles and in the oviduct. In vivo LPS challenge increased the expression of several *AvBDs* in the theca tissue. In contrast, in the cultured theca tissue, LPS upregulated the expression of *IL1β* and *IL6*, but did not affect the *AvBDs* expression; whereas IL1β upregulated the expression of the AvBD12 gene and protein. It suggests that LPS of Gram-negative bacteria are recognized by TLR4 to induce the IL1β expression, and then IL1β upregulates the AvBD expression in the infected tissues. The expression of *AvBDs, IL1β* and *IL6* in the vagina was upregulated by *Salmonella* and LPS challenge. Meanwhile, the *AvBD1* and *3* expression was upregulated by IL1β, suggesting that the synthesized IL1β may participate to induce AvBDs. The results of this study suggest that innate immune system with TLRs and AvBDs is developed in the chicken gastrointestinal tract and reproductive organs. Improvement of innate immune functions by AvBDs in chickens may be one of the important strategies for enhancing the disease resistance in chickens.

Keywords: Immunodefense, Gut, Reproductive organs, Antimicrobial peptides

INTRODUCTION

Maintenance of animal health is essential to obtain their maximum productivity and safe products. The intestine and reproductive organs could be susceptible to various bacterial and viral pathogenic agents. *Salmonella* organisms in feed that may enter the chicken gastro-intestinal tract are one of the most common risk factors responsible for food-borne diseases in humans. They may be incorporated by macrophages in the intestinal mucosa, and may be transferred to the ovary and oviduct. The contamination of eggs by *Salmonella typhimurium* may also lead to vertical infection and increase the mortality of embryo and chicks. The tropical environment may cause heat stress that affects the immune functions of animals through the endocrine system of the hypothalamus, pituitary and adrenal gland. Global warming may facilitate this problem in animal production. The usage of antibiotic agents must be regulated for the food safety. We expect the enhancement of innate immune functions is an important strategy to improve the disease resistance of animals.

Avian β-defensins (AvBDs) are the member of antimicrobial peptides involved in the innate immune system. Fourteen AvBD genes have been identified in chicken (AvBD1 to AvBD14) (van Dijk *et al*., 2008), and they are believed to kill a wide spectrum of microorganisms including Gram-

positive and Gram-negative bacteria, protozoa as well as some fungi and enveloped viruses. Thus, they may have efficacy to protect the tissues from infection by unforeseen pathogenic microbes. The innate immune response including expression of AvBDs and cytokines is initiated by the interaction of pathogen-associated molecular patterns (PAMPs) of microbes with their recognition receptors (PRRs). Toll-like receptors (TLRs) are the primary receptors that recognize PAMPs. In chickens, the TLR1 to -5, -7, -15 and -21 have been identified (Brownlie and Allan, 2011). TLR2 forms a heterodimer with TLR1 and recognizes peptidoglycan, lipoteichoic acid and lipoprotein of the Gram-positive bacterial cell wall. TLR3 and TLR4 recognize the double-stranded RNA of infectious viruses and lipopolysaccharide (LPS) of Gram-negative bacteria such as *Salmonella* and *E. coli*, respectively. TLR5 and TLR7 interact with bacterial flagellin and single-stranded RNA of viruses, respectively. TLR15, a unique chicken TLR, recognizes the non-secreted, heat-stabile component of both Gram-negative and Gram-positive bacteria, and secreted virulence-associated fungal and bacterial proteases. TLR21 recognizes unmethylated CpG oligo-DNA of microorganisms.

The aim of this study was to characterize the innate immune system with the focus on the expression of AvBDs in the digestive and reproductive organs for the future strategy to enhance the disease resistance of chickens.

Identification of TLRs and AvBDs expressed in the gastrointestinal tract of broiler chicks

The expression of all TLRs (TLR1.1 and 1.2, TLR2.1 and 2.2, 3, 4, 5, 7, 15, and 21) was identified in both the proventriculus and cecum of Chunky broiler chicks. A total of 7 AvBDs (AvBD1, 2, 4, 6, 7, 10, and 12) were identified in the proventriculus and 8 AvBDs (AvBD1, 2, 4, 5, 6, 7, 10, and 12) were in the cecum, respectively. Thus, it is suggested that a variety of PAMPs of microbes are recognized by different TLRs, probably leading to regulate the synthesis of innate immune factors including AvBDs. The immunoreactive (ir)-AvBD12 was localized in the surface epithelium and the cells in the connective tissues of proventricular glands. In the chicks given with probiotics for 2 weeks, the expression level of AvBD12 in the proventriculus was not different between probiotics group and control chicks, whereas the ir-AvBD12 density in the surface epithelium was significantly lower in probiotics chicks than in control chicks. These results suggest that, although probiotics-feeding does not affect the gene expression of AvBDs, it may stimulate AvBD12 secretion from the surface epithelium of the proventriculus in broiler chicks.

Expression of TLRs and AvBDs in the hen ovary

We have identified the expression of TLR2, 4, 5 and 7 in the theca layer, and TLR4 and 5 in the granulosa layer of hierarchal follicles of laying hens. In the study of Michailidis *et al*. (2010), the hen ovary expressed TLR1.2, 2.1, 3 to 5, 7, 15 and 21. We have shown that experimentally inoculated *Salmonella enteritidis* were localized in the theca and granulosa layers. It is possible that the LPS, flagellin and CpG-ODN of *Salmonella* bacteria are recognized by these TLR4, 5 and 21 in the ovarian and follicular tissues. We have also identified the expression of 6 AvBDs in the theca and 4 AvBDs in the granulosa layer of follicles. Injection of birds with LPS increased the expression of several AvBDs in the theca tissue. In contrast, LPS upregulated the expression of IL1β and IL6, but did not affect the expression of AvBD10 and -12; whereas IL1β upregulated the expression of the AvBD12 gene and protein in cultured theca tissue. It is assumed that LPS of Gram-negative bacteria are recognized by TLR4 to induce the expression of IL1β, and then IL1β upregulates the AvBD expression in the infected tissues. Thus our results suggest that innate immune system to recognize PAMPs through TLRs and to synthesize proinflammatory cytokines and AvBDs are developed in the ovarian follicles. This innate immune system may play roles in the local immunodefense against infection by Gram-negative bacteria including *Salmonella* bacteria in the follicles.

Expression of TLRs and AvBDs in the hen oviduct

Salmonella organisms phagocytized by macrophages in the intestine may be transported not only to the ovary but to the oviduct through the blood stream and colonize there. Also, microorganisms colonizing the cloaca may ascend the oviduct through the vagina and uterus. Thus, the immune system in the vagina play important role to protect the oviduct from infection. The studies of our group and Michailidis *et al*. (2011) identified the expression of all types of TLR in the oviduct of laying hens.

The expression of 11 AvBDs was identified in the oviduct of laying hens. The expression of AvBDs, IL1β and IL6 in the vagina was upregulated by *Salmonella* infection and by LPS challenge. Stimulation by poly(I:C) (viral dsRNA), flagellin (flagellum of bacteria) and CpG-ODN (microbial DNA), which are the ligands of TLR3, 5 and 21, upregulated the expression of IL1β and IL6, but not AvBDs in the cultured uterus or vaginal mucosal cells. Meanwhile, the expression of AvBD1 and 3 was upregulated by IL1β in the cultured vaginal cells. Thus, the synthesized proinflammatory cytokine, IL1β, may participate to induce AvBDs. It is suggested that the innate immune system composed of TLRs, proinflammatory cytokines and AvBDs plays roles in the mucosal defense against pathogenic microbes in hen oviduct.

We have identified the ir-AvBD3 on the surface of fibers forming the outer layer of the eggshell membrane, and ir-AvBD3, -11 and -12 in the eggshell matrix. These AvBDs are probably secreted from the cells in the isthmus or uterus to the eggshell membrane and eggshell. It is assumed that these AvBDs play roles also in protection of eggs from invading microbes.

CONCLUSION

Pathogenic microbes often appear in the poultry farm, and appearance of unforeseen microbes may be increased under the global warming environment. Improvement of innate immune functions by AvBDs in chickens may be one of the important strategies to prevent the infection by them since AvBDs are believed to have antimicrobial activity to a wide spectrum of microbes. The current study confirmed the AvBDs synthesis in the gastro-intestinal and reproductive organs, whereas they could be expressed in the other organs. Breeding and feeding management such as probiotics may be considered for the strategy to improve their expression.

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