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«Review»

Biomolecules Triggering Altered Food Intake during Pathogenic Challenge in Chicks

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Food intake is regulated by several complicated synergistic mechanisms that are affected by a variety of internal and external influences. Some of these factors include those that are released from pathogens such as bacteria, fungi, and viruses, and most of these factors are associated with suppression of the chick's food intake. Although chicks are well-known to decrease their food intake when they experience a pathogenic challenge, the mechanisms that mediate this type of satiety are poorly understood. One of the goals of our research group has been to better understand these mechanisms in chicks. We recently provided evidence that pathogen-associated molecular patterns, which are recognized by pattern-recognition receptors such as Toll-like receptors, likely contribute to satiety in chicks that are experiencing a pathogenic challenge. Additionally, we identified several inflammatory cytokines, including interleukin-1β, tumor necrosis factor-like cytokine 1A, prostaglandins, and nitric oxide, that likely contribute to satiety during a pathogenic challenge. This review summarizes the current knowledge on pathogen-induced satiety in chicks mainly accumulated through our recent research. The research will give good information to improve the loss of production during infection in poultry production in the future.

Key words: chick, feeding, infection, pathogen-associated molecular patterns, Toll-like receptor

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Introduction

Chicks are precocial immediately after hatching and search for food even when their down is still wet from the egg. Thus, chicks hatch with an exceptionally well-developed neural circuitry that causes them to actively search for and ingest food. Appetite regulation in any vertebrate, including chickens, is complicated and is affected by a wide variety of internal and external factors, including age, reproductive status, food availability and palatability, environmental temperature, and exposure to stressors, among many other influences.

Pathogen challenge has a significant effect on food intake in chickens. In general, chicks with a high pathogen load exhibit greatly reduced food intake, resulting in production loss. Therefore, studying the mechanisms that cause reduced food intake in chickens with high pathogen loads may provide novel insights to mitigate production and profitability losses. When pathogens such as bacteria, fungi, and viruses invade, the chicken immune system is triggered to eliminate or neutralize these pathogens. In addition, infections are frequently associated with non-specific symptoms such as weight loss, pain, fatigue, malaise, and loss of appetite. The chicken immune system is activated when it encounters components of pathogens, including pathogen-associated molecular patterns (PAMPs). In vertebrates, PAMPs bind to pattern recognition receptors, including Toll-like receptors (TLRs). PAMPs are associated with non-specific symptoms in the host, including anorexia, hypoactivity, hyperthermia, and changes in the digestive function in mammals.

It is therefore possible that PAMPs are a trigger to reduce food intake in chickens with a pathogen load. However, when we started this area of research, there was very little knowledge regarding the effect of PAMPs in chickens. Therefore, one focus of our research group has been to understand how PAMPs reduce food intake in chicks. This review summarizes the findings of our ongoing research.

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Table 1. **Summary of human TLRs and their ligands.**

TLR	Ligand (PAMPs)	Ligand source	Cell localization
TLR1	Triacyl lipopeptides	Bacteria	Cell surface
TLR2	Peptidoglycans	Bacteria	Cell surface
	LTA	Gram-positive bacteria	
	Zymosan	Fungi	
TLR3	Double-stranded RNA	Viruses	Cell compartment
	Poly I:C	Synthetic compound	
TLR4	LPS	Gram-negative bacteria	Cell surface
TLR5	Flagellin	Bacteria	Cell surface
TLR6	Diacyl lipopeptides	Mycoplasmas	Cell surface
TLR7	Single-stranded RNA	RNA viruses	Cell compartment
	Imidazoquinoline	Synthetic compound	
	Resiquimod	Synthetic compound	
	Imiquimod	Synthetic compound	
TLR8	Single-stranded RNA	RNA viruses	Cell compartment
TLR9	Unmethylated oligo-DNA containing CpG motif	Bacteria, DNA viruses	Cell compartment
TLR10	Triacyl lipopeptides	Bacteria	Cell surface

Abbreviations: TLR, Toll-like receptor; PAMPs, pathogen-associated molecular patterns; LTA, lipoteichoic acid; poly I:C, polyinosinic-polycytidylic acid; LPS, lipopolysaccharide; CpG, unmethylated cytosine-guanine dinucleotide.

TLRs and PAMPs

TLRs

TLRs are members of the pattern-recognition receptor group that bind to molecules frequently found in pathogens, including PAMPs. In vertebrates, TLRs are expressed in several cell types, including dendritic cells, macrophages, and epithelial cells. In humans, 10 TLRs, namely TLR1–10, have been isolated to date (Table 1) (Kawasaki and Kawai, 2014). Although mice lack a functional TLR10 gene, they have three other TLRs not found in humans: TLR11, TLR12, and TLR13 (Kawasaki and Kawai, 2014). TLRs are categorized into two subfamilies according to their subcellular localizations: one subfamily is expressed on the cell surface and the other is expressed intracellularly in organelles, including the endosome.

Cell-surface TLRs include TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10, whereas intracellular TLRs include TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13 (Kawasaki and Kawai, 2014). Cell-surface TLRs recognize microbial membrane components, including lipopolysaccharide (LPS), lipoproteins, and proteins, whereas intracellular TLRs recognize nucleic acids associated with bacteria and viruses. Most TLRs function as homodimers, whereas TLR2 forms a heterodimer with TLR1 or TLR6. Most TLRs are associated with the adaptor protein myeloid differentiation factor 88 (MyD88), except TLR3, which only has TIR-domain-containing adapter-inducing interferon-β (TRIF) as its adaptor.

Chickens also have TLRs, and several reviews focusing on chicken TLRs are available (Kannaki et al., 2010; Brownlie and Allan, 2011; Nawab et al., 2019). Several TLRs have been identified in chickens (TLR1, TLR2, TLR3, TLR4, TLR5, and TLR7) (Fukui et al., 2001; Iqbal et al., 2005; Yilmaz et al., 2005), and their ligands are similar to those observed in mammals (Kannaki et al., 2010; Brownlie and Allan, 2011; Nawab et al., 2019). In addition to them, chickens have unique TLRs, namely TLR15 and TLR21. Chickens lack TLR9, whose ligand is unmethylated oligo-DNA containing CpG motif of bacteria and viruses; however, chickens TLR21 has a similar function to mammalian TLR9 (Brownlie et al., 2009). TLR15 appears to be unique to birds, as it has not been isolated in mammals (Kannaki et al., 2010; Brownlie and Allan, 2011; Nawab et al., 2019). Chicken TLR15 recognizes fungal and bacterial proteases (de Zoete et al., 2011; Boyd et al., 2012). Additionally, there are two subtypes of TLR1 and TLR2 in chicken. Thus, chicken TLRs have evolved differently from those of mammals, but maintain their original function of recognizing and activating the innate immune system, as is the case in mammals.

TLR4 and LPS

TLR4 is a well-known receptor for LPS (Table 1), which is the most well-studied PAMP in vertebrates because it induces a robust immune response against bacterial infection. LPS is an endotoxin found in the cell wall of gram-negative bacteria and is released when the cell disintegrates. LPS comprises an O-polysaccharide, a core oligosaccharide, and lipid A, and induces strong immunoreactivity and non-specific symptoms in vertebrates. In rodents, LPS induces anorexia, hypoactivity, hypothermia, or fever, and decreases gastric emptying (van Miert and De la Parra, 1970; Langhans et al., 1990; Kozak et al., 1994; Kanra et al., 2006).

The amino acid sequence of chicken TLR4 has moderate ho-

Table 2. **Effects of PAMPs on behavioral and physiological parameters in chickens.**

PAMPs	Injection route	Behavioral and physiological parameters				References
		Feeding	Cloacal temperature	Crop emptying	Corticosterone release	
LPS	IP	Decreased	Increased	Decreased	Increased	Johnson et al. (1993a) Johnson et al. (1993b) Tachibana et al. (2016) Tachibana et al. (2017b)
	ICV	Decreased	Increased	–	Increased	
LTA	IP	No change	–	–	–	Tachibana et al. (2016)
	ICV	No change	–	–	–	
Zymosan	IP	Decreased	Increased	Decreased	Increased	Tachibana et al. (2020a) Tachibana et al. (2021b) Takahashi et al. (2021)
β-glucan	IP	Decreased	–	No change	–	
Mannan	IP	No change	–	No change	–	
Chitin	IP	No change	–	No change	–	
Flagellin	IP	Decreased	Increased	Decreased	Increased	Tachibana et al. (2021a)
Poly I:C	IP	Decreased	No change	No change	Increased	Tachibana et al. (2019b) Tachibana et al. (2022c)
	ICV	Decreased	No change	Decreased	Increased	
Resiquimod	IP	Decreased	Decreased	Decreased	Increased	Tachibana et al. (2020b) Tachibana et al. (2022b)
Imiquimod	IP	No change	No change	No change	No change	

Abbreviations: PAMPs, pathogen-associated molecular patterns; LPS, lipopolysaccharide; LTA, lipoteichoic acid; Poly I:C, polyinosinic-polycytidylic acid; IP, intraperitoneal injection; ICV, intracerebroventricular injection; –, not investigated

mology to that of mammalian TLR4 (42% and 51% homology to the extracellular and cytoplasmic domains of mouse TLR4, respectively) (Yilmaz et al., 2005). Chicken *TLR4* mRNA is highly expressed in the cecal tonsils, macrophages, and heterophils (Iqbal et al., 2005). Moderate expression of chicken *TLR4* mRNA has been observed in several organs, including the digestive tract, spleen, and liver (Iqbal et al., 2005). LPS is the most well-investigated PAMP in birds. Similar to mammals, LPS not only activates the innate immune system but also induces behavioral and physiological changes in birds (Table 2). Johnson et al. (1993a, 1993b) were the first to extensively study LPS in chickens. They found that intraperitoneal (IP) injection of LPS decreased food intake, induced somnolence, increased body temperature, and increased the plasma corticosterone (CORT) concentration (Johnson et al., 1993a). A subsequent study showed that *TLR4* mRNA is not only distributed in the peripheral organs but also in the brain (Iqbal et al., 2005). Johnson et al. (1993a) also found that both IP and intracerebroventricular (ICV) injections of LPS decreased food intake. The anorexigenic effect of LPS following ICV injection has also been demonstrated by other research groups, including our own (Zendehdel et al., 2012; 2016; Tachibana et al., 2016). However, to our knowledge, it remains unclear whether LPS can pass the blood–brain barrier in chickens. Artunkal et al. (1977) suggested that young chicks have not yet established a functional blood–brain barrier; therefore, LPS easily accesses the brain. Additionally, LPS has been shown to disrupt the blood–brain barrier in mammals (Varatharaj and Galea, 2017). It is therefore possible that blood-borne LPS directly affects neural activity in the brains of chicks.

Additionally, we found that IP injection of 100 μg LPS obtained from *Escherichia coli* O127 decreased the feed passage rate through the crop (i.e., crop emptying rate) in chicks (Tachibana et al., 2017b). Moreover, LPS administration caused conditioned visual aversion in chicks, suggesting that LPS induces an aversive sensation (Tachibana et al., 2022b). Changes in feed passage through the digestive tract and the aversive sensation may contribute to the anorexigenic effect of LPS.

TLR2 and its Ligands

TLR2 has a variety of ligands, including peptidoglycan, lipoteichoic acid (LTA), and zymosan (Table 1). In chickens, two types of TLR2 have been identified: TLR2A (TLR2 type 1) and TLR2B (TLR2 type 2) (Fukui et al., 2001). Both types of TLR2 have moderate amino acid sequence homology to mammalian TLR2 (TLR2A, 41% and 74%; TLR2B, 44% and 74% homology to the extracellular and cytoplasmic domains of mouse TLR2, respectively) (Yilmaz et al., 2005). The chicken *TLR2A* and *TLR2B* genes are expressed in several organs, including the spleen, cecal tonsil, and digestive tract, but the expression of *TLR2B* is stronger than that of *TLR2A* (Iqbal et al., 2005). *TLR2B*, but not *TLR2A*, is expressed in the kidney, brain, testes, heart, and macrophages (Iqbal et al., 2005).

LTA is a component of gram-positive bacterial cell walls, and its IP injection (from *Bacillus subtilis*) induces non-rapid eye movement sleep, hypothermia, hyperthermia, hypoactivity, and anorexia in mice (Szentirmai et al., 2021). Thus, LTA is considered to induce non-specific symptoms during gram-positive bacterial infections in mammals. However, neither IP nor ICV injection of LTA from *Streptococcus pyogenes* affects food intake in chicks (Table 2) (Tachibana et al., 2016). The reason for this lack of effect of LTA on feeding in chicks remains unknown, al-

though the source of LTA differed between studies (Tachibana et al., 2016; Szentirmai et al., 2021). Currently, there is no evidence that LTA induces non-specific symptoms, including anorexia, in chicks.

Zymosan is a PAMP derived from the cell wall of yeast, which is primarily composed of polysaccharides such as β-glucan, chitin, and mannan (Di Carlo and Fiore, 1958). As an agonist of TLR2, zymosan causes severe inflammation and multiple organ dysfunction syndromes by stimulating various inflammatory mediators (Volman et al., 2005). Furthermore, IP injection of zymosan induces hypophagia and hyperthermia in rodents (Cremeans-Smith and Newberry, 2003; Naoi et al., 2006; Hubschle et al., 2007; Saito and Watanabe, 2008; Bastos-Pereira et al., 2014; 2017). Zymosan treatment increased reactive oxygen species levels in chicken blood phagocytes, including heterophils and macrophages, *in vitro* (Desmidt et al., 1996; Merrill et al., 1996; Guabiraba et al., 2017). In addition, IP injection of zymosan induced leukocyte infiltration into the abdominal cavity and stimulated reactive oxygen species production in chickens (More Bayona et al., 2017). We found that IP injection of 2.5 mg zymosan derived from *Saccharomyces cerevisiae* suppressed feeding behavior in chicks (Table 2) (Tachibana et al., 2020a). This dose of zymosan also increased the cloacal temperature and reduced the crop emptying rate in chicks (Tachibana et al., 2020a; 2021b; Takahashi et al., 2021). Interestingly, zymosan components such as β-glucan, chitin, and mannan had little or no effect on the feeding behavior and crop emptying rate in chicks (Tachibana et al., 2020a; 2021b). This indicates that a combination of these components, or other unknown components, is required to exert the effect of zymosan in chicks. Similar to LPS, IP injection of zymosan causes conditioned visual aversion in chicks (Tachibana et al., 2022b). The mechanism underlying the effect of zymosan may be similar to that of LPS.

Fukui et al. (2001) reported that chicken TLR2B failed to respond to *S. cerevisiae* and zymosan *in vitro*. Thus, it is possible that the effect of zymosan is not mediated by TLR2 in chickens. In mammals, the complement system and macrophages are affected by zymosan (Nieuwenhuijzen et al., 1993; Miller et al., 1996). Therefore, zymosan might exert its effect through the complement system and/or macrophages rather than via TLR2 in chicks.

TLR5 and Flagellin

TLR5 recognizes flagellin, a protein component of the bacterial flagella (Table 1) (Hayashi et al., 2001). Chicken TLR5 has moderate amino acid sequence homology with that of mammals (47% and 60% homology to the extracellular and cytoplasmic domains of mouse TLR5, respectively) (Yilmaz et al., 2005), and is moderately expressed in several organs, including the digestive tract, spleen, cecal tonsil, bursa, lung, liver, kidney, testis, and heart (Iqbal et al., 2005).

Mice injected IP or intravenously (IV) with 10–100 μg flagellin showed increased plasma concentrations of proinflammatory cytokines, including interleukin (IL)-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and nitrate, by activating inducible nitric oxide synthase (iNOS) (Eaves-Pyles et al., 2001; Liaudet et al., 2002). An IV injection of a higher dose (10 mg/kg) of flagellin induced hypotension, a reduction in vascular contractility, and death in mice (Eaves-Pyles et al., 2001). Furthermore, flagellin injection reduced wheel-running and body weight gain in mice (Matsumoto et al., 2008), suggesting that flagellin and TLR5 are also related to physiological and behavioral changes in vertebrates.

Flagellin activates the chicken immune system. The IP injection of flagellin increases peripheral blood leukocyte concentrations, especially heterophils, in chickens (Genovese et al., 2007). Furthermore, flagellin stimulates the mRNA expression of *IL-1β* and *IL-6* in chicken heterophils (Kogut et al., 2006). In addition, we found that IP injection of 10 μg flagellin decreased the food intake and crop emptying rate in chicks (Table 2) (Tachibana et al., 2021a). The effect of flagellin on body temperature is biphasic; IP injection of flagellin first tends to decrease cloacal temperature, followed by an increase (Tachibana et al., 2021a). However, these effects were not observed when flagellin-22, a 22-amino acid sequence fragment of the conserved N-terminal part of flagellin that activates plant defense mechanisms (García and Hirt, 2014), was injected into chicks (Tachibana et al., 2021a). Thus, other parts of flagellin, including the C-terminal domain, may play an important role in inducing non-specific symptoms in chicks.

Most studies in chickens have focused on LPS rather than on other bacteria-derived PAMPs. In summary, the results accumulated to date regarding flagellin suggest that flagellin, similar to LPS, is associated with non-specific symptoms during bacterial infection.

TLR3 and Poly I:C

TLR3 is distributed in the cellular components, but not in the cell membrane, and recognizes double-stranded RNA (Table 1). The amino acid sequence of chicken TLR3 shows moderate homology to that of mice (57% and 67% homology to the N-terminal and C-terminal domains of mouse TLR3, respectively) (Yilmaz et al., 2005). Chicken TLR3 is highly expressed in the digestive tract, cecal tonsil, liver, and kidney (Iqbal et al., 2005).

Polyinosinic-polycytidylic acid (poly I:C) is a synthetic double-stranded RNA that binds TLR3 and is widely used as a viral mimetic. In mammals, poly I:C injection affects locomotor activity and induces anorexia, fever, and stress responses. More specifically, IP injection of poly I:C decreases locomotor activity in mice (Cunningham et al., 2007; Zhu et al., 2016) and cage activity and wheel running activity in rats (Hopwood et al., 2009). ICV injection of poly I:C also decreased locomotor activity in mice (Zhu et al., 2016), and both ICV and IP injections induced anorexia in mice (Zhu et al., 2016) and rats (Hopwood et al., 2009). IV, IP, and ICV injection of poly I:C induces fever in mice (Cunningham et al., 2007; Zhu et al., 2016), rabbits (Kimura et al., 1994), and rats (Bastos-Pereira et al., 2015). In addition, IP injection of poly I:C increases the plasma CORT concentration in mice (Guha-Thakurta and Majde, 1997).

Although poly I:C induces cytokine gene expression in the

spleens of chickens (St Paul et al., 2012), as observed in mammals (Guha-Thakurta and Majde, 1997), very little is known about its effects on behavioral and physiological responses. We demonstrated that IP injection of 400 μg poly I:C reduced food intake in chicks, but had no effect on cloacal temperature and the crop emptying rate (Table 2) (Tachibana et al., 2019b). Since *TLR3* is expressed in the chicken brain (Iqbal et al., 2005), we hypothesized that poly I:C might directly act on the brain to induce anorexia in chicks. We found that ICV injection of 10 and 40 μg poly I:C reduced food intake in chicks and suppressed the crop emptying rate, whereas it had no effect on cloacal temperature (Tachibana et al., 2019b).

TLR7 and Resiquimod

In mammals, TLR7 and TLR8 are considered to respond to infection of single-stranded RNA viruses (Uematsu and Akira, 2006). Activation of TLR7 by its agonists induces the expression of several cytokines such as ILs, IFNs, and TNF-α (Dockrell and Kinghorn, 2001). In addition, TLR7 is considered to mediate the physiological and behavioral changes that occur during viral infection, based on evidence that intranasal administration of the TLR7 agonist SM360320 (1V136) induced anorexia, adipsia, hypothermia, and hypoactivity in mice (Hayashi et al., 2008). In addition, subcutaneous and IP injections of imiquimod, a TLR7 agonist, induced anorexia and hyperthermia in rats (Damm et al., 2012). The discrepancy in the response of body temperature to TLR7 agonists between studies could be due to the difference in experimental conditions such as animal species and injection route. Regardless of these differences, TLR7 agonists appear to affect feeding behavior, body temperature, and activity in mammals. Furthermore, TLR7-induced anorexia and decreased physical activity were found to be mediated via TNF-α and/or prostaglandin E2 (PGE2) production (Oyanagi et al., 2018).

TLR7 is present in birds, whereas TLR8 appears to have been lost during evolution (Philbin et al., 2005; Iqbal et al., 2005; Yilmaz et al., 2005; MacDonald et al., 2008). Chicken TLR7 has moderate amino acid sequence homology to mammalian TLR7 (61% and 63% homology to the extracellular and cytoplasmic domains of mouse TLR7, respectively) (Yilmaz et al., 2005). The chicken *TLR7* gene is highly expressed in the spleen and moderately expressed in several peripheral tissues, including the cecal tonsils (Iqbal et al., 2005; Philbin et al., 2005). Furthermore, single-stranded RNA and resiquimod (R848, a synthetic TLR7 ligand) could induce the gene expression of *IL-1β, IL-6,* and *IL-8* in chicken splenocytes (Philbin et al., 2005). Imiquimod also induced the gene expression of *IL-1β, IL-6*, and *INF-α* in duck splenocytes (MacDonald et al., 2008). Thus, peripheral TLR7 plays an important role in the response of birds to single-stranded RNA viruses.

However, little is known about the effects of TLR7 on other physiological responses in birds. We demonstrated that IP injections of 25 and 100 μg resiquimod reduced food intake in chicks (Table 2) (Tachibana et al., 2020b). The same doses of resiquimod also decreased the cloacal temperature and crop emptying rate (Tachibana et al., 2020b). However, imiquimod, another synthetic TLR7 ligand, had no effect on these behavioral and physiological parameters in chicks at the same doses (Tachibana et al., 2020b). This effect was further supported by the work of Dockrell and Kinghorn (2001), who showed that resiquimod alters the gene expression of cytokines more strongly than imiquimod in mammals.

Bioactive Molecules

Feeding Regulation Peptides

For several decades, many groups have investigated peptides expressed in the brains of chicks that regulate food intake, and more than 40 candidate peptides have been identified. Neuropeptide Y (NPY) and agouti-related peptide (AGRP) are representative orexigenic peptides, and α-melanocyte-stimulating hormone [derived from proopiomelanocortin (POMC)] and corticotropin-releasing hormone (CRH) are representative anorexigenic peptides in chicks (Tachibana and Tsutsui, 2016). Since the mRNA expression of *TLR1, TLR2B, TLR3, TLR4*, and *TLR5* is observed in the brain of chickens (Iqbal et al., 2005), these TLRs may be associated with some of these feeding regulation peptides and contribute to PAMP-induced anorexia. Based on this hypothesis, we investigated the effect of IP injection of 100 or 200 μg LPS on the gene expression of the aforementioned feeding regulation peptides in the diencephalon of chicks. We expected that LPS would decrease the gene expression of orexigenic peptides and increase that of anorexigenic peptides. However, injection of 200 μg LPS significantly increased *NPY* gene expression and 100 μg LPS significantly decreased *POMC* gene expression (unpublished data). These changes are considered to be compensatory: increased *NPY* mRNA levels might cause the chick to start eating again after the LPS-induced anorexia effect decays, and the LPS-induced anorexia effect may be so strong that it causes a decrease in the expression of other anorexigenic factors such as POMC. In addition, *AGRP* expression in the diencephalon was not affected by any dose of LPS (unpublished data). CRH is part of the hypothalamus-pituitary-adrenal axis, which releases glucocorticoids from the adrenal glands. Although LPS stimulates CORT release in chickens (Johnson et al., 1993a), we found that it had no effect on *CRH* gene expression in the diencephalon of chicks (unpublished data). Additionally, LPS did not affect the expression of other feeding regulation peptides such as growth hormone-releasing hormone, ghrelin, and urocortin-3 in the diencephalon (unpublished data).

Thus, our results did not support the hypothesis that these feeding regulation peptides are associated with infection-induced anorexia in chicks. Therefore, other bioactive molecules with immune system functions may be candidates for mediating infection-induced anorexia. It is also possible that other feeding regulation factors that we have not evaluated or that have not yet been identified are responsible for these effects.

Cytokines

Cytokines are bioactive low-molecular-weight proteins that function as immune-modulating agents via autocrine, paracrine, and endocrine signaling. Given the widely accepted evidence

that PAMPs induce proinflammatory cytokines, it is conceivable that these cytokines are associated with the anorexia induced by PAMPs. Indeed, several proinflammatory cytokines such as IL-1, IL-6, IL-8, IFN-α, IFN-γ, and TNF-α have been shown to suppress feeding behavior in rodents (Plata-Salamán et al., 1988; Bodnar et al., 1989; Langstein et al., 1991; Crnic and Segall, 1992; Plata-Salamán and Borkoski, 1993; Reyes-Vázquez et al., 1994; McCarthy, 2000; Plata-Salamán, 2001). Burgess et al. (1998) demonstrated that IL-1β-deficient mice did not exhibit reduced food intake following ICV injection of LPS, although food intake was reduced following IP injection of LPS. IP injection of antiserum to IL-6 also attenuated the anorexigenic effect of subcutaneous LPS injection, whereas antisera to IL-1β and TNF-α had no effect (Harden et al., 2006). Furthermore, the combination treatment of an IL-1 receptor antagonist, a monoclonal antibody to IL-6, and a TNF-binding protein fragment completely abolished the anorexigenic effect of LPS in mice (Swiergiel and Dunn, 1999). Thus, proinflammatory cytokines likely play an important role in infection-induced anorexia in mammals.

These proinflammatory cytokines are also induced by PAMPs in chickens (Philbin et al., 2005; Kogut et al., 2006; MacDonald et al., 2008; Takahashi et al., 2008; St Paul et al., 2012; Zhang et al., 2013). Our previous studies revealed that splenic mRNA expression levels of *IL-1β, IL-6, IL-8*, and *IFN-γ* were increased by LPS, zymosan, flagellin, poly I:C, and resiquimod in chicks (Tachibana et al., 2018a; 2019b; 2020a; 2021b). These PAMPs also increased the mRNA expression of tumor-necrosis factor-like cytokine-1A (*TL1A*), an avian homologue of mammalian *TNF*, in chicks (Tachibana et al., 2018a; 2019b; 2020a; 2021a). However, the effects of these cytokines on food intake in chickens are different from those observed in mammals. ICV injection of IL-1β decreased food intake in chicks, whereas IP injection of this cytokine had no effect (Tachibana et al., 2017a). IV and ICV injections of TL1A also reduced food intake in chicks (Takimoto et al., 2005; Tachibana et al., 2018a). However, ICV injection of IL-6, IL-8, IFN-α, and IFN-γ did not affect food intake in chicks (Tachibana et al., 2017a; 2018a). Thus, the role of these cytokines in infection-induced anorexia differs between chicks and rodents.

Prostaglandins

Prostaglandins are bioactive lipid compounds that are synthesized from arachidonic acid by cyclooxygenase (COX). There are two isoforms of COX: COX-1 is constitutively expressed in various tissues, whereas the expression of COX-2 is induced by inflammation. In mammals, prostaglandins are associated with non-specific symptoms during infection. IP injection of LPS increases the plasma and cerebrospinal fluid concentrations of PGE2 and prostaglandin D2 (PGD2) in rats (Gao et al., 2009). LPS treatment also increases the production of PGD2 and prostaglandin F2α (PGF2α) in rat Kupffer cells (Brouwer et al., 1995). Central and peripheral injections of PGE2 and PGF2α have been associated with reduced food intake (Scaramuzzi et al., 1971; Levine and Morley, 1981) and affect gastric emptying in rats (Ruwart and Rush, 1984; Stein et al., 1994). Furthermore, LPS-induced anorexia and inhibition of gastric emptying were attenuated by indomethacin, an inhibitor of COX, in rodents (Langhans et al., 1989; Calatayud et al., 2002; Liang et al., 2005). These results suggest that prostaglandins are involved in the effects of LPS in mammals.

Prostaglandins also appear to be associated with the effect of PAMPs in chickens given that IV injection of LPS increased the plasma PGE2 concentration in 5-week-old chickens (de Boever et al., 2010). Johnson et al. (1993b) demonstrated that pretreatment with an IP injection of indomethacin, a COX inhibitor, attenuated LPS-induced anorexia in chickens. In addition, IP and ICV injections of indomethacin attenuated LPS-induced hyperthermia and drowsiness (Johnson et al., 1993b). Our preliminary experiments also revealed that IP injection of 100 or 200 μg LPS increased *COX-2* gene expression in the diencephalon of chicks (unpublished data). Thus, prostaglandins are considered to play important roles in the behavioral and physiological changes that occur during infection in chicks. However, the specific prostaglandins that mediate these effects remain unclear.

We found that both ICV and IP injections of PGE2 and PGF2α reduced food intake in chicks (Tachibana et al., 2017c). We also found that ICV injection of PGD2 suppressed food intake in chicks (Tachibana et al., 2018b). Among these prostaglandins, PGE2 has a more potent anorexigenic effect comparing with PGD2 and PGF2α. This suggests that PGE2 may play a crucial role in inhibiting food intake under infectious conditions. Interestingly, the effect of PGD2 in chicks is opposite to that in mammals; ICV injection of PGD2 reduced food intake in chicks and increased food intake in rodents (Ohinata et al., 2008).

Nitric Oxide (NO)

NO is a biologically active gaseous molecule synthesized by the enzyme NOS, which converts L-arginine to L-citrulline. NO is involved in a wide range of biological functions, including vasodilation, blood pressure, penile erection, intestinal peristalsis, food intake, and inflammation (Aisaka et al., 1989; Morley and Flood, 1991; Konturek and Konturek, 1995; Förstermann and Sessa, 2012). There are three isoforms of NOS: neuronal NOS, endothelial NOS, and iNOS (Förstermann and Sessa, 2012).

Among the three isoforms of NOS, the expression of iNOS is induced by proinflammatory cytokines and PAMPs (Förstermann and Sessa, 2012). It is therefore expected that iNOS and its product, NO, are associated with the immune responses induced by pathogens. In fact, LPS-associated mortality is prevented in iNOS-knockout mice (Wei et al., 1995). NO has also been associated with LPS-induced anorexia, adipsia, fever, decreased physical activity and energy expenditure, and reduced gastric emptying in guinea pigs and rats (Roth et al., 1998; Inada et al., 2006; Riediger et al., 2010).

In chicks, IP injections of zymosan, flagellin, poly I:C, and resiquimod increased the plasma concentrations of NO_2^- and NO_3^- (NOx, metabolites of NO) (Takahashi et al., 2021; Tachibana et al., 2021a; 2022c). Moreover, *iNOS* mRNA expression could be induced by several PAMPs, including zymosan, poly I:C, and resiquimod (Takahashi et al., 2021; Tachibana et al., 2022c).

Since the iNOS inhibitor S-methylisothiourea (SMT) abolished the zymosan-, poly I;C-, and resiquimod-induced increase in plasma NOx concentrations (Takahashi et al., 2021; Tachibana et al., 2022c), it can be concluded that these PAMPs induce iNOS, thereby increasing NO production in chicks. Moreover, IP and ICV injections of sodium nitroprusside, an NO donor, significantly reduced food intake in chicks (Takahashi et al., 2022). These results imply that PAMPs stimulate NO production and that NO reduces food intake during times of infection. However, co-injection of SMT with IP did not attenuate the anorexigenic effects of poly I:C and resiquimod (Tachibana et al., 2022c). Therefore, it is unlikely that NO directly mediates the anorexigenic effects of PAMPs.

Other Factors

Histamine is another candidate factor that mediates anorexigenic effects during times of infection. Zendehdel et al. (2016) demonstrated that a histamine H1 receptor antagonist attenuated the reduction in food intake induced by ICV injection of LPS in young chickens. This result suggests that central histamine and H1 receptor are associated with LPS-induced anorexia. Furthermore, it has been demonstrated that ICV and IP injections of histamine significantly decrease food intake in chicks (Kawakami et al., 2000; Tachibana et al., 2019a). Compound 48/80, a stimulator of mast cells, also suppressed food intake in chicks following ICV and IP injections (Kawakami et al., 2000; Tachibana et al., 2019a), suggesting that mast cells release histamine during times of infection, which then contributes to reduced food intake.

Bradykinin, a biologically active peptide member of the kinin group, consists of nine amino acids and is involved in mammalian inflammation. Ornithokinin is an avian homologue of mammalian bradykinin (Kimura et al., 1987). The amino acid sequence of ornithokinin is RPPGFTPLR, which differs from that of mammalian bradykinin, where T^6 and L^8 are substituted for L^6 and F^8, respectively (Kimura et al., 1987). Bradykinin and ornithokinin are both synthesized from precursor kininogens by the action of kallikrein. Guabiraba et al. (2017) found that inoculation with avian pathogenic *E. coli* increased the mRNA expression levels of hepatic kininogen and B1 receptors in chickens, indicating that avian ornithokinin is related to infection. We recently demonstrated that both IP and ICV injections of ornithokinin cause reduced food intake in chicks (Tachibana et al., 2022a), suggesting that ornithokinin is associated with infection-induced anorexia. However, in our experiment, IP injection of LPS had no effect on kininogen mRNA expression, but decreased the liver kallikrein mRNA expression level in chicks (Tachibana et al., 2022a). Therefore, the role of ornithokinin in infection-induced anorexia should be further investigated in the future.

Zendehdel et al. (2012) showed that SB242084 and DL-AP5, which are antagonists of the 5-hydroxytryptamine (5-HT, serotonin) 2C receptor and the N-methyl-D-aspartate receptor (a glutamate receptor), respectively, attenuated anorexia induced by ICV injection of LPS in chicks. This study also suggested that 5-HT and glutamate are associated with the anorexigenic effect of LPS.

Future Studies Required

As noted earlier, we demonstrated that PAMPs likely trigger the anorexia induced by bacterial, fungal, and viral infections in chicks. In addition, a variety of immune-related bioactive molecules such as proinflammatory cytokines, prostaglandins, and NO have been found to reduce food intake in chicks. Since PAMPs induce the proinflammatory cytokine expression and synthesis of prostaglandins and NO, these bioactive molecules might mediate the effect of PAMPs. However, the specific bioactive molecules that play crucial roles in infection-induced anorexia should be clarified in the future. Among these bioactive molecules, prostaglandins are the strongest candidates for mediating anorexia because Johnson et al. (1993b) showed that pretreatment with a COX inhibitor attenuated LPS-induced anorexia in chickens.

Food intake is regulated by several organs, including the brain. Since the anorexigenic effects of PAMPs are observed after IP injection in chicks, peripheral PAMPs likely access the brain or initiate a pathway that results in brain changes. Clarification of this pathway will likely provide a novel perspective on the mechanisms contributing to anorexia during infection.

PAMPs not only induce anorexia but also reduce the crop emptying rate and are associated with the development of conditioned aversion in chicks, which may contribute to anorexia. In addition, animals are frequently drowsy and hypoactive during infections. Thus, future studies should be performed to link aspects of behavior, physiology, histology, and molecular biology to clarify the precise mechanism underlying anorexia during times of infection.

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Author Contributions

Tetsuya Tachibana: study design, concept, data collection, analysis and manuscript writing

Mark A Cline: study design, concept, manuscript writing and review

Conflicts of interest

The authors declare no conflict of interest associated with this manuscript.

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