Mini Review

Infectious Bursal Disease Virus-Induced Chicken Innate Immune Genes Expression

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Abstract | Infectious bursal disease virus (IBDV) belongs to the genus *Avibirnavirus*, a dsRNA virus that causes an acute, highly contagious and immunosuppressive disease in young chickens. The first line of defence against the virus is innate immunity whose activation is independent of the antigen and depends on the ability of the host to recognize pathogens by means of specific pattern recognition receptors. This interaction induces the production of cytokines, chemokines and interferons. Through Janus kinase/signal transducers and transcription activators pathway, interferon induces the transcription of interferon-stimulated genes (ISGs), responsible for playing an essential and decisive role in the virus pathology. Some studies have already reported the overexpression of these genes against IBDV infection in susceptible cells. The protection ability of ISGs to safeguard chicken cells against the virus could be potentially applied for vaccine production, better vaccination protocols and infection control in the future.

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The International Committee for the Taxonomy of Viruses (ICTV) recognizes eight distinct families of dsRNA (double stranded ribonucleic acid) viruses, including viruses with medical, veterinary or agricultural significance. Majority of these viruses have icosahedral capsid structures and have the genome composed of two dsRNA segments (Mertens, 2004).

Infectious bursal disease virus (IBDV) is a dsRNA virus and is a major cause of economic losses in the poultry industry. This virus belongs to the family *Birnaviridae*, genus *Avibirnavirus* (ICTV, 2015). Since the virus causes a highly contagious disease especially in young chickens known as infectious bursal disease (IBD) or Gumboro disease, it is included in the OIE list of diseases. The virus causes severe immune-suppression by targeting the precursors of antibody producing B cells in the bursa of Fabricius, leading to depletion of B cells and atrophy of the primary immune organ (Wang et al., 2010).

There are two serotypes (1 and 2), however, serotype 2 does not induce lesions in bursa, whereas serotype 1 is pathogenic to susceptible birds. Turkeys, ducks

and ostriches may be naturally and experimentally infected, however infections are apatogenic. The domestic chickens are the only avian species susceptible to clinical disease. The virus is highly stable and has a tendency to persist in the environment despite application of thorough cleaning and disinfection (Sharma et al., 2000). Serotype 1 strains of IBDV replicate efficiently in lymphoid cells of the bursa of Fabricius of chickens whereas strains of serotypes 1 and 2 are widely propagated in chicken embryo fibroblast cells (CEF). Several cell lines, including Vero (African green monkey kidney cells), CER (chicken embryo related cells), LSCC-BK3 (chicken B lymphoblastoid cells), LSCC-HD11 (chicken macrophage-like cells), Hi-5 (cabbage looper ovary cells), Sf9 (Spodoptera frugiperda pupal ovarian tissue cells) and DF-1 (a continuous cell line of chicken embryo fibroblast) can be used for virus propagation, however, the DF-1 and CEFs are proven to be as susceptible to IBDV infection (Lin et al., 2007).

The first-line of defence against pathogens is the innate immune response in vertebrate species. Recognition of potential pathogens by the innate immune system is the function of pattern-recognition receptors (PRRs) which detect pathogen-associated molecular patterns (PAMPs) for induction of effector molecules. In chickens, ten different Toll-like receptors (TLRs), a well-characterized member of PRRs, have been identified and their natural or synthetic ligands representing PAMPs have been identified (Alkie and Rautenschlein, 2015).

The interaction between PRRs and PAMPs activates the signalling pathways that initiate microbicidal mechanisms, the production of pro- and/or anti-inflammatory cytokines, and upregulation of co-stimulatory molecules required for antigen presentation to the acquired immune system (Kogut et al., 2006).

Although B cells are the principal targets, recent data show that IBDV also infects and replicates in macrophages, implicating in cytokines and pro-inflammatory production, which is correlated with the extensive inflammatory response in the bursa. In addition, macrophages also constitute a major component of innate immunity against infection, particularly against virus infections (Khatri and Sharma, 2006).

Rasoli et al. (2015) found a similar expression of immune genes in response to *in vitro* infection of HD11 cells, and in bursa and spleen following *in vivo* infection. Messenger RNA (mRNA) expression levels of the pro-inflammatory cytokine, IL-1β, the pro-inflammatory chemokines CCL4, CXCLi1 and CXCLi2, the Th1 cytokines IL-12α and IL-18 and iNOS (inducible nitric oxide synthase) were all upregulated in very virulent IBDV (vvIBDV)-infected HD11 cells. For MHC class I, mRNA expression levels were generally upregulated. This is an expected immune response to viral infection. The innate immune response is triggered, followed by a Th1 response leading to increase in viral antigen presentation in the context of MHC class I, and the production of effector molecules, such as NO through the induction of iNOS. This result is similar as previously reported by Khatri et al. (2005) where IBDV in adherent macrophage cell isolated from bursa of IBDV challenged chicken. In contrast, mRNA expression levels of the anti-inflammatory cytokine IL-10 were generally down-regulated. Of the TLRs measured, only the mRNA expression levels of TLR3, which recognises dsRNA as IBDV, were significantly altered post-infection, with levels increasing with time post-infection. MHC class II mRNA expression levels were down-regulated, as were those of the anti-inflammatory cytokine IL-10, indicating that the macrophage-like cells were switched to a strong Th1-promoting anti-viral phenotype for the HD11 in vitro model.

Khatri and Sharma (2006) have demonstrated that *in vitro* infection of macrophages with IBDV results in an increased expression of iNOS, COX-2 and IL-8 transcripts. However, the addition of p38 and NF-κβ, pharmacological inhibitors specific for the two important pathways, resulted in a blockade of the expression of iNOS, COX-2 and IL-8, suggesting that IBDV uses these pathways to elicit macrophage activation.

Lee et al. (2014) determined the chicken melanoma differentiation-associated gene 5 (MDA5) recognizes infectious bursal disease virus infection and initiates and amplifies an innate immune response in the chicken MDA5 (chMDA5) signalling pathway. Knockdown and overexpression of chMDA5 were performed by transfecting infected DF-1 cells. The mRNA expression levels of chicken MDA5, IRF-7, IFN-beta, PKR, OAS, Mx and MHC class I in IBDV-infected DF-1 cells exhibited significant upregulation. The expression levels of chicken MDA5, IRF-7, IFN-beta and MHC class I in chMDA5-knockdown DF-1 cells were significantly lower compared to con-

trol. DF-1 cells overexpressing chMDA5 showed significantly higher expression of chicken MDA5, IRF-7, IFN-beta, PKR, OAS, Mx and MHC class I.

Autophagy is also an essential component of the host innate immunity. Hu et al. (2015) reported the binding of the pathogen receptor HSP90AA1 (Heat Shock Protein 90 Alpha Family Class A Member 1) to the viral protein VP2 of IBDV induces autophagy by inactivating the AKT-MTOR (mechanistic target of rapamycin) pathway in early infection of DF-1 cells.

Therefore, the interaction of IBVD surface antigens with host receptors initiates the transcription of innate immunity genes responsible for the direct or indirect effects of interferons and proinflammatory mediators, playing an essential and decisive role in the virus pathology. Exploring the protection ability of these genes to safeguard chicken cells against the virus could be potentially applied in favour of vaccine production, better vaccination protocols and infection control in the future.

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Conflict of Interest

All authors declare that they have no conflict of interest.

Authors' Contribution

Conceptualization: CWA, HLF, Data curation: GMN, HLF, Formal analysis: GMN, HLF, CWA, Investigation: GMN, HLF, CWA, Methodology: GMN, HLF, CWA, Project administration: CWA, HLF, Funding acquisition: CWA.

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