Review Article

Innate Immune Responses Against Avian Respiratory Viruses

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Abstract | The mucosal surfaces of respiratory, intestinal and reproductive tracts are the primary entry points for viruses into host cells. Amongst these, respiratory tract is prone to direct and continuous exposure to viral infection. Respiratory viruses including influenza virus, coronaviruses and herpes viruses are important pathogens that cause significant losses in the poultry industry. However, successful establishment of infection in the respiratory tract switch on the pathogen recognition by innate immune receptors and establish first line of defense against viral infections. When specific cellular receptors recognize a virus they trigger the translation of inflammatory cytokine and chemokines leading to establishment of an antiviral state of the host. In this review, we discuss the recent understanding in host responses to avian viral infection and their impact in health and disease.

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Introduction

Eukaryotes respond to viral infection with a remarkable defense system known as the immune system. Immune system comprises of an innate, complement, adaptive or specific defenses. The innate immune system is categorized as the first line of immune defense, because it became of its nature of activation in response to infection. Innate immunity succeeds to clear or halt a number of viral infections due to prompt response just within minutes to hours after infection. Cells that cover outer surface of respiratory tract, intestinal internal lining and genital tracts, are the prime sites for entry of a broad range of viruses. Of particular importance is the respiratory tract, which is under direct and continuous contact to the external environment. Due to continuous exposure to pathogens, lungs and airways are easy targets for airborne infections. In fact, most of the respiratory viruses are sensed promptly and eradicated by sophisticated signaling program of the innate and adaptive immune system of the respiratory tract. However, respiratory viruses of chicken, such as influenza virus, infectious bronchitis virus (IBV), Newcastle disease virus and infectious laryngotracheitis virus (ILTV), are still potential threat and are able to establish acute infections in the upper respiratory tract.

A crucial function of innate immune system is to recognize specific conserved microbial structures of pathogens as 'foreign' particles, known as pathogen associated molecular patterns (PAMPs). PAMPs are indispensable for existence, infectivity and broadly shared by microorganisms. These PAMPs such as viral

proteins and nucleic acids are recognizable by specific kind of cellular receptors, known as pattern recognition receptors (PRRs). PRRs have diverse molecular bases which capture antigen and activate downstream signaling pathways (Michael et al., 2012). There are three classes of PRRs, retinoic acid-inducible gene I (RIG-I)-like helicases (RLH) (Fensterl and Sen, 2009), Toll-like receptors (TLR) (Pang and Iwasaki, 2011) and cytosolic DNA sensors including absent in melanoma 2 (AIM2) and cyclic GMP-AMP synthase (cGAS), (Mansur et al., 2014) which can regulate the transcriptional expression of interferons (IFN)s and inflammatory cytokines. Upon viral attachment, PRRs recruit downstream signaling molecules and activate IFN regulatory factors 3 (IRF-3) and 7 (IRF-7) directly or indirectly, as well as activating protein 1 (AP-1) and nuclear factor kappa B (NF-κB) (Goraya et al., 2015; Santhakumar et al., 2017). Activation of these molecules is essential to trigger the transcription of type I IFN genes and set up an antiviral state by triggering cascade of hundreds of IFN-stimulated genes (ISGs) in the infected cells.

In this review we discuss important avian respiratory viruses, including influenza virus, infectious bronchitis virus and infectious laryngotracheitis virus. We consider the involvement of these pathways for major avian respiratory infections. Both, single-stranded RNA (ssRNA) viruses (e.g. avian influenza virus) and double-stranded DNA (dsDNA) such as ILTV virus are continuous threat to poultry industry. The severe pulmonary disease caused by these viruses illustrates the potentially detrimental impact of an overly robust innate inflammatory response. êHere, we discuss recent advances in our understanding of antiviral immunity in the lungs, from the initiation of innate and adaptive responses following viral infection.

Innate immune recognition of infection via PRRs

The most efficient PRRs are the TLRs, which consist of fifteen members in mammalians. TLRs are type I proteins of PRRs family and are critical for discriminating between self and non-self-molecules. TLRs are highly conserved in almost all type of species, including mammals, fish, amphibians, insects and birds (Roach et al., 2005). TLRs bind with viral nucleic acid and proteins followed by conveying downstream signals to form a complex with adapters and kinases that lead to activation of type I IFNs and several other antiviral cytokines. TLRs of chicken have specific properties and they use cytoplasmic Toll interleukin-1 receptor (TIR) domain to pass cellular signals through the molecules like, myeloid differentiation primary response 88 (MyD88), TRIF and TRAM (O'Neill et al., 2003). In case of respiratory viruses, TLR3 and TLR7 recognize intermediate and final products of viral replication dsRNA and ssRNA, respectively (Lund et al., 2003; Schlender et al., 2005; Parvizi et al., 2012). Fascinatingly, chicken do not have viral DNA sensor TLR9, while in mammals viral DNA is sensed by TLR9. However, TLR-mediated DNA sensing is mediated by a functional ortholog TLR21, which is absent in human (Roach et al., 2005; Santhakumar et al., 2017).

TLRs that recognize nucleic acids in the cell are located in late endosomes. That helps to optimize the binding capacity of TLRs to recognize the viral nucleic acids and limit their accession to host self-derived nucleic acids (Heil et al., 2003; Matsumoto et al., 2003). Whereas TLRs expressed in endosome (TRL3, 7 and TLR8) or expressed on the cell surface (TLR1, 2, 4 or 6), start different signaling pathways, to trigger the transcription of IFN inducible genes (Boehme and Compton, 2004). êTLR2 involves the recruitment of TIR domains, TIR domain binds along with adaptor protein/MyD88 (TIRAP/Mal). TIRAP-MyD88 complex initiate same response as by TLR7/8 (Fig. 1) (Jensen and Thomsen, 2012). However TLR8 is non-functional in chicken, so chicken genome encode an extra protease cleavage dependent TLR15 to be functional (de Zoete et al., 2011). As TLR8 is non-functional and TLR9 is lacking in chicken, RNA viruses only recognized by TLR3/7 (Santhakumar et al., 2017). Not only the genomic variations but functional differences also exist in TLR-mediated viral recognition in chickens. TLRs, except TLR3, signal via Myd88, TLR3 signaling initiated by binding with TRIF (TIR-domain-containing adapter-inducing interferon-β), TRIF stimulates the serine-threonine kinases IκKε (IKKε) and TBK1, through phosphorylation of the IRF3, which enters the nucleus and induces expression of the IFNβ gene (reviewed (Goraya et al., 2015). Other TLRs bind with MyD88 and recruit TLR-binding TIR domain and two interleukin-1 receptor associated kinases (IRAKs), IRAK4 and IRAK1 (Figure 1). Upon activation, IRAK1 binds with TNF receptor-associated factor 6 (TRAF6), and dissociates from the TLRs to initiate following activation of IFNs. Both TLRs de-

pendent signaling pathways terminate in the translation of type I IFNs (Jensen and Thomsen, 2012).

RIG-I receptors interact with 5'-triphosphate containing RNA and initiate the important signals for early cytokine induction in response to infection with RNA viruses (Yoneyama et al., 2004; Hornung et al., 2006). Melanoma differentiation-associated gene 5 (MDA5), which recognizes long dsRNA, is crucial for innate immune recognition of avian viruses (Kato et al., 2006; Chen et al., 2016). Whereas RIG-I is absent in chickens and other members of the order Galliformes (Karpala et al., 2011). Despite lacking RIG-I, chickens respond to RNA viruses and mount effective type I IFN responses, most possibly due to chicken MDA5 and LGP2, chicken MDA5 (Figure 1) can sense both long and short dsRNA (Liniger et al., 2012; Hayashi et al., 2014). It is known that chicken LGP2 similar to human RIG-I, is an end binder, whereas chicken MDA5 is a stem binder of dsRNA (Uchikawa et al., 2016). Downstream signaling pathways utilized by RNA helicases is similar to TLRs signaling, which ultimately activate IRF and NF-κB for the production of IFNs (Le Goffic et al., 2007). The strategic difference between RNA helicases and TLRs is that the RNA helicases are contained throughout the cytosol, rather than being limited to intracellular compartments. Therefore, the viruses that infect cells by direct membrane fusion and without entering the endosome, still, can trigger innate immune signaling through RNA helicases.

In addition to above molecules there are several DNA sensors in cells to sense cytosolic DNA, either non-self DNA or DNA from nuclear/mitochondrial damage. There are several proteins which act as DNA sensors, but major cytosolic sensors of DNA are PYHIN family member AIM2 and cGAS. However, several

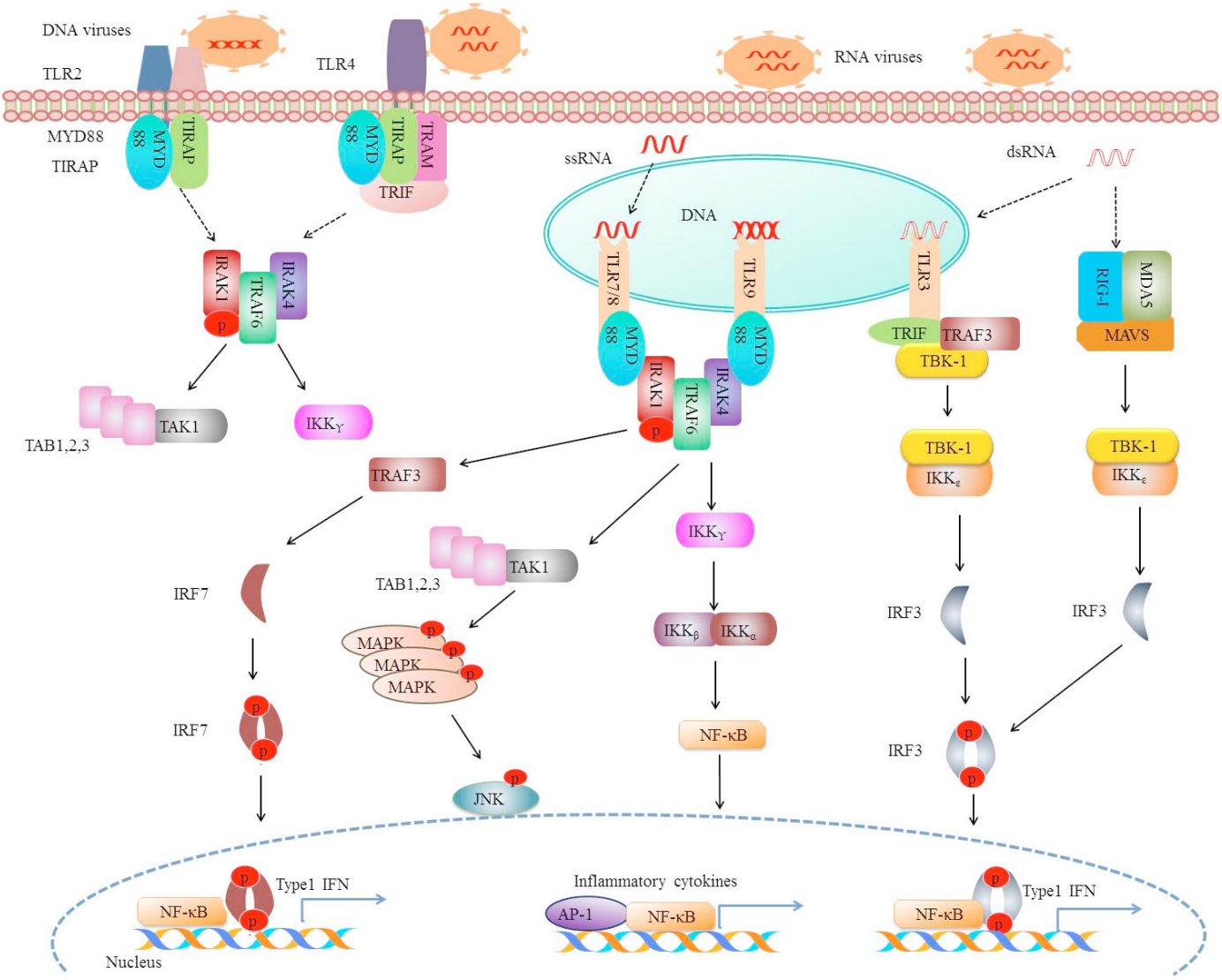

Figure 1: *Sensing of DNA and RNA respiratory viruses by different pathogen recognition receptors and downstream signaling for the production of interferon to establish antiviral state.*

other proteins also have been recognized as DNA receptors, which include Z DNA binding protein 1 (ZBP1/DAI), the helicase DDX41, and IFI16 (Cavlar et al., 2012; Veeranki and Choubey, 2012). Downstream of these DNA sensors, the stimulator of IFN genes (STING) acts as an adapter and stimulates type I IFN production through the similar pathway as by TLRs and RLH by triggering IRF3 and NF-κB transcription factors activation (Schlee and Hartmann, 2016). Although DNA sensors in chickens has not yet been studied in greater detail, but it is known that chicken STING can actively sense cytosolic DNA and in cooperation with the mitochondrial antiviral-signaling protein activates the innate immune responses independent of RIG-I, interfering with the replication of RNA viruses. In chicken RLH support the activation of STING mediated type I IFN induction (Cheng et al., 2015).

Respiratory Viruses

Influenza virus

Influenza virus is a member of the family Orthomyxoviridae, and is a negative sense ssRNA virus. The enveloped viral genome consists of eight segments of ssRNA, which is tightly wrapped by nucleoprotein (NP). Haemagglutinin (HA) and neuraminidase (NA) are viral glycoproteins which play vital role in subtyping of influenza virus and effectively sensed by antibodies. êThese proteins also play their role in the host determination and reproduction of the virus. The HA initiates helps the virus to bind with the host cell via binding with the corresponding receptors (sialic acid) present on the cell surface. Whereas the NA protein improves the export of new virus by budding to the cell surface and helps in spread of virus. IAV has maintained two feature in poultry, first is HA receptor binding specificity for α 2,3 sialic acid while in case for humans it is adopted to bind 2,6 sialic acid receptors. The second one is deletion of amino acids from the NA stalk region (Blok and Air, 1982). Influenza virus outbreaks can be seasonal or pandemic depending on genetic mutations. The frequent genetic mutations of influenza A virus cause vaccine failure and challenge the ultimate eradication of influenza virus (Shao et al., 2017). Back in date avian flu was known as fowl plague.

Birds infected with influenza virus show high response of innate immune genes including higher levels of cytokine and severe pulmonary pathology with marked cellular infiltrates (Kobasa et al., 2007; Burggraaf et al., 2014). Avian influenza A (H5N1) strains had showed capacity to induce intense cytokine and inflammatory responses in birds and human with high mortality rate (de Jong et al., 2006). áIn chicken, TLR3 and TLR7 have been conventional to interact with influenza virus in specific cell populations. The viral recognition activates the cellular helicases, RIG-I and MDA5 and/or toll like receptors (TLRs), arbitrate the recruitment of downstream signaling proteins, which end up with activated IFN regulatory factor 3 (IRF3) and nuclear factor kappa B (NF-κB) transcription factors. Addition to ssRNA detection, RIG-I and MDA5 play key role by sensing the vRNP, transcriptional intermediates with 5'triphosphate and dsRNA while viral replication, which are different in molecular structure of cellular RNA (Pichlmair et al., 2006). When viral 5'triphosphate or dsRNA attached with the repressor domain (RD) of RIG-I, conformational changes occur to expose caspase activation and recruitment domains (CARDs). The tripartite motif containing protein 25 (TRIM25) ubiquitinate RIG-I involved domains and activate the signaling (Munir, 2010). Later activated RIG-I associate with MAVS; (also known as IPS-1, VISA or Cardif), and leads to the activation of IRF3 and NF-κB. RIG-I receptors role in the activation of NF-κB have been extensively reviewed (Loo and Gale, 2011; Thaiss et al., 2016). NS1 suppress the ubiquitination of the RIG-1 by inhibiting the function of TRIM25 which is essential step in RIG-1 signaling (Rückle et al., 2012). TLRs particularly sense RNA viruses, TLR3 and TLR7 binds with the dsRNA like and ssRNA, such as influenza virus respectively. When viral RNA binds with TLRs, it activates the signaling of proinflammatory cytokines, chemokines and IFN production with the help of cellular adaptor proteins (Alexopoulou et al., 2001). Binding with influenza RNA activates TLR7, activated TLR7 interacts with downstream adopter protein MyD88 in plasmacytoid dendritic cells (DCs), specialized for IFN production. This leads to the activation of IRF7 and NF-κB, and trigger high levels of IFNβ and IFNα transcription (Lund et al., 2004). TLR-3 signaling starts binding with TRIF (TIR-domain-containing adapter-inducing interferon-β), TRIF activates the serine-threonine kinases IκKε (IKKε) and TBK1, through phosphorylation of the transcription factor IRF3, which enters the nucleus and initiate IFN production. In short endosomal TLRs and RIG-I have pivotal roles in influenza virus infection. TLR3 induces an antiviral state

by recognition of infected cells with the detrimental effect of recruiting damage-inducing inflammatory cells, whereas TLR7 induces IFN responses to block viral replication and also promote antibody responses. However due to high mutation rate and immune evasion through several viral mechanisms, make influenza virus to establish infection successfully.

Infectious bronchitis virus

Infectious bronchitis virus (IBV) has a single-stranded positive sense RNA, belongs to the family *Coronaviridae*. Chicken is the primary natural host of IBV; however other avian species like geese duck and pigeon may have important role to spread the IBV strains throughout the world (Awad et al., 2014). IBV primarily targets the epithelial cells of the respiratory, urinary and reproductive tracts in the domestic poultry (Cook et al., 2012). IBV infects almost all ages of chicken results in severe respiratory problems and causes high mortality up to 30% in chicks less than 4 weeks old and egg production loss in layers with high economic losses. Being an ssRNA virus, IBV is highly susceptible to mutation especially in the spike protein gene, which is attached to membrane glycoprotein (M) on the surface of the virus particles. The S protein comprise of two subunits S1 and S2, though S1 subunit recognized as PAMP and causes the release of neutralizing and hemagglutination inhibiting (HI) antibodies (Ignjatovic and Galli, 1995).

After challenging with IBV an increase in TLR-3 mRNA expression observed in number of studies, and its function to trigger immune response is well established (Kameka et al., 2014; Okino et al., 2017). In another study, induction of TLR-1 LA, TLR-1 LB, TLR-2, TLR-3, and TLR-7 gene was significantly up-regulated in the tracheal epithelial cells of 21day old chicks, immunized with attenuated IBV Massachusetts (IBV-Mass) by intranasal inoculation (Guo et al., 2008). In response to infection with IBV an innate immune response occurs in pulmonary tissues and viral RNA recognized by TLR3 and TLR7. Activation of TLR7 leads to recruitment of MyD88 adopter protein leading to transcription of pro-inflammatory cytokines and IFNs. The nucelocapsid (N) protein helps virus to evade viral immune response to interfere with the 2', 5' Oligoadenylate synthetase/RNaseL (2',5' OAS) activation, which is responsible for the induction of Type I IFN, N protein can also inhibit the production of several proinflammatory cytokines and chemokines via interacting with induction of IFN (Kameka et al., 2014; Okino et al., 2017). IBV can trigger the MAPK signaling pathways as well, those involved in the interferon response (Smith et al., 2015). The non-structural protein 3 (Nsp3) encoded by coronaviruses plays a role in deubiquitinization activity, which inhibits nuclear translocation of the IRF3 and interfere sequential translation of Type I IFNs (Clementz et al., 2010). In another study, severe acute respiratory syndrome coronavirus (SARS-CoV) and IBV spike protein of both coronaviruses can interact with host eukaryotic initiation factor 3 (eIF3), which is responsible for the modulation of host immune gene expression (Xiao et al., 2008).å The chicken TLR-21 is functionally similar to TLR9 in Mammals, and after stimulation with de-oxyoligonucleotides containing CpG motifs, it induces the production of NFκ-B and leads to enhanced expression of several cytokines (Brownlie et al., 2009). It is found that treatment of deoxyoligonucleotides containing CpG motifs prior to infection with IBV helps to restrict the viral growth in chicken embryos by enhancing the translation of IFNβ and other cytokines (Dar et al., 2009). The innate immune response against IBV is not enough to draw a conclusion for control of disease, though in recently lot of studies has been published regarding innate immunity has sparked further interest. There are continuous mutations occur in antigenic S1 protein and new IBV genotypes emerging globally. Currently live vaccines are the solution to develop broader protection against IBV variants. To fully control the disease we have to develop new strategies by expanding our understandings of the immune response to IBVs.

Infectious laryngotracheitis virus

Infectious laryngotracheitis virus (ILTV) is a double stranded DNA virus and is member of the alphaherpesvirinae subfamily. The viral glycoproteins are important as major antigens inducing antibody and cell mediated immune responses glycoproteins, namely glycoprotein (g) B, gC, gD, gE, gG, gH, gI, gJ, gK, gL and gM (Fuchs et al., 2007) and are responsible for virus entry and replication in the host. ILTV can be transmitted horizontally, and mainly infects the upper respiratory tract of chicken, including conjunctival and tracheal mucosa (Oldoni et al., 2009). After getting entry into the cells ILTV causes short lytic infection in upper respiratory epithelial cells and latent infection in neurons of the trigeminal ganglia. Innate immune response has been recognized as important in the protection against disease. The airways mucous

is a first barrier that virus must break before contact with the respiratory epithelial cells. In the innate immunity, TLRs present on immune cells which recognize the specific PAMPs are gaining increasing attention in disease control. However there is little known about the innate immune responses against ILTV.

Notably, the cell surface harbor TLR2 that is the predominant plasma membrane localized receptor involved in antiviral defense. TLR2 is expressed by antigen presenting cells (APC) and non-immune cells, such as mucosal epithelial cells (Iwasaki 2010). It is well established that TLR2 can recognized herpes and other DNA viruses including HSV, Epstein Barr virus and varicella-zoster virus (Boehme et al., 2006; Szomolanyi-Tsuda et al., 2006; Rathinam and Fitzgerald, 2011). TLR2 work together with co-receptors TLR1 or TLR6, recognize the herpes virus glycoproteins like gB and gH and leads to activation of NF-κB (Boehme et al., 2006). The hetrodimerization of TLR1-TLR2 is considered to instigate the dimerization of intracellular TIR domain. Later, the TLR2-TLR1 complex triggers downstream signaling cascade via MAL and MyD88 adaptor proteins that causes the activation of NF-κB and MAP kinases and ultimately start the transcription of IFN and other cytokines (Jin et al., 2007). Lipoteichoic acid (LTA) induced TLR2 cluster of differentiation 14 (CD14) signaling pathway and induced antiviral responses against ILTV infection via up regulating of innate proteins such as MyD88, iNOS and IL-1β (Haddadi et al., 2015). Following the interaction with cell membrane viruses enter into the cell and recognized by number of TLRs localized in endosome. In mammalian cell TLR9 recognize the DNA viruses while in chicken TLR21 is functionally similar to TLR9 and expressed in endosomal compartments. TLR9 can recognize un-methylated CpG-rich DNA that is frequently present in the genomes of microbes unlike to host cells. In a study, Thapa and co-workers observed that CpG DNA restrict the ILTV replication in lungs and stimulate the macrophages. CpG DNA increases the expression of IL-1β, NO and mRNA expression of iNOS. Finally, CpG DNA elicits immune responses against ILTV infection and decreased the mortality and morbidity (Thapa et al., 2015). ILTV infection can trigger the expression of many immune and cell signaling related genes including IL-6, IL-8, IL-15, CXC K60, CCL17 and CCL20 (Lee et al., 2010). Still, the detailed mechanism of innate response against ILTV infection has not been examined very well. Studies are required to understand the detailed sensing of viral DNA and role of viral protein to evade the immune mechanism.

Conclusions

The nature of the challenge posed by viruses in poultry is continuously changing as a result of pathogen evolution and environmental changes. A number of viruses prefer to infect birds via mucosal membranes and these viruses in wild and commercial birds are causing zoonotic epidemics and huge economic losses to commercial industry respectively. In order to develop novel therapeutics to control avian respiratory diseases, including novel vaccines or vaccine adjuvants, it is imperative to identify disease resistance genes and how pathogens can sabotage these responses. Recent advances in innate immunity has elucidated molecules and molecular pathways involved in innate immune response against avian respiratory viruses. The activation of the innate immunity appears in response to high pathogenesis and mortality related to these viruses. While PRRs are critical in the activation of these responses, characterizing the role of RNA helicases and DNA sensors has recently been appreciated. As we target several cellular genes of innate immunity (such as siRNA and CRISPR/Cas9) and molecular drugs to alter the signaling pathways, these studies will be important in our future clinical approach, vaccine development to control these viruses.

Author's Contributions

Mohsan Ullah Goraya design and write the main text of study, Liaqat Ali and Iqra Younis helped to collect the literature draw the figure and manuscript writing.

Conflict of interest

All the authors declare that there is no conflict of interests regarding the publication of this article.

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