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Immune responses to infectious laryngotracheitis virus

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ABSTRACT

Infectious laryngotracheitis (ILT) is an upper respiratory tract disease in chickens caused by infectious laryngotracheitis virus (ILTV), an alphaherpesvirus. Despite the extensive use of attenuated, and more recently recombinant, vaccines for the control of this disease, ILT continues to affect the intensive poultry industries worldwide. Innate and cell-mediated, rather than humoral immune responses, have been identified as responsible for protection against disease. This review examines the current understandings in innate and adaptive immune responses towards ILTV, as well as the role of ILTV glycoprotein G in modulating the host immune response towards infection. Protective immunity induced by ILT vaccines is also examined. The increasing availability of tools and reagents for the characterisation of avian innate and cell-mediated immune responses are expected to further our understanding of immunity against ILTV and drive the development of new generation vaccines towards enhanced control of this disease.

1. Background

Infectious laryngotracheitis (ILT) is an upper respiratory tract disease of chickens caused by infectious laryngotracheitis virus (ILTV, *Gallid herpesvirus 1*), a member of the *Alphaherpesvirinae* sub-family (genus *Iltovirus*) (Davison, 2010). Like other members of this family, ILTV has an icosahedral nucleocapsid, double stranded genomic DNA (Fuchs et al., 2007) and the capacity to establish asymptomatic life-long latent infections (Bagust, 1986; Williams et al., 1992).

Infectious laryngotracheitis virus is transmitted horizontally, and primarily infects the upper respiratory tract of susceptible chickens, including conjunctival and tracheal mucosa (Bagust et al., 1986; Oldoni et al., 2009). The highest viral titre in these tissues occurs between 4 and 6 days following experimental inoculation (Bagust et al., 1986; Kirkpatrick et al., 2006; Oldoni et al., 2009). During the initial viral replication period, ILTV is detected in the trigeminal ganglia having been found in this tissue as early as 2 days after experimental inoculation (Bagust et al., 1986; Oldoni et al., 2009). Viral DNA has also been detected in caecal tonsils and cloaca of experimentally inoculated chickens (Oldoni et al., 2009). In addition, colonisation of liver in 20% of 3-day-old chickens has been described, indicating systemic spread of the virus (Bagust et al., 1986). Although viraemia has never been described, the capacity of ILTV to infect leucocytes (Chang et al., 1977) and more specifically, macrophages (Calnek et al., 1986) *in vitro* has been suggested as a mechanism for systemic spread of the virus to non-respiratory sites (Oldoni et al., 2009).

Latency is established in respiratory tissues. This has been demonstrated by tracheal organ cultures obtained from latently infected birds in which reactivation of infection and viral shedding was observed in focalised points of the trachea (Bagust, 1986). The involvement of trigeminal ganglia during latent infection was only confirmed by PCR in 1992 (Williams et al., 1992). Viral reactivation associated with certain stressors such as re-housing with unfamiliar birds or onset of lay has also been reported (Coppo et al., 2012a; Hughes et al., 1989, 1991). Putative latency associated transcripts (LATs) have been detected within the major transcriptional activator infected cell protein 4 (ICP4) (Johnson et al., 1995), Two micro RNAs (miRNA) also map to ICP4 (Waidner et al., 2009), and down regulate ICP4 expression by directly cleaving the ICP4 transcript. Authors speculate that regulation of ICP4 expression via miRNAs could impact the balance between lytic and latent states, aid in maintaining latent infections *in vivo* or play a role in viral reactivation (Waidner et al., 2011).

Typical clinical signs during lytic infection include conjunctivitis with mucous or serous ocular discharge, coughing, dyspnoea, gasping and mortality in severe cases. Decreased egg production and/or weight gain can accompany these clinical signs. Typically,

Abbreviations: CEO, chicken embryo origin; DIVA, differentiation of infected and vaccinated animals; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; gB, glycoprotein B; gG, glycoprotein G; IBDV, infectious bursal disease virus; IBV, infectious bronchitis virus; ICP4, infected cell protein 4; IL2, interleukin-2; IL18, interleukin-18; ILT, infectious laryngotracheitis; ILTV, infectious laryngotracheitis virus; HVT, herpes virus of turkeys; miRNA, microRNA; TCO, tissue culture origin; vCKBP, viral chemokine binding protein.

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enzootic forms of the disease present with high morbidity (90–100%) and variable mortality (5–70%) that is usually around 10–20% (Devlin et al., 2011; Guy and García, 2008). An increase in body temperature accompanies the infection, with highest temperatures observed between 4 and 6 days after exposure. Leukopenia, with differential white blood cell counts showing lymphopenia and heterophilia, has been observed between 3 and 7 days post-inoculation (Chang et al., 1977). Gross pathological findings include serous, mucous, bloody or even diphtheritic exudates in tracheal or conjunctival mucosa (Guy and García, 2008; Tripathy, 1998). Microscopically, the lesions are characteristic of herpesvirus infection with formation of multinucleated cells and eosinophilic intranuclear inclusion bodies, detectable from day 3 after experimental inoculation (Hayashi et al., 1985). Oedema and inflammatory cell infiltration can be observed in the *lamina propria* of affected mucosa (Guy and García, 2008; Guy et al., 1990; Tripathy, 1998). Together with the assessment of gross and microscopic pathological findings, the disease can be diagnosed by direct identification of the virus in biological samples collected from infected birds. Viral isolation has commonly been used for detection of ILTV (Tripathy, 1998), however, now molecular methods such as PCR are routinely used (Callison et al., 2007; Diallo et al., 2010; Mahmoudian et al., 2011). Notably, molecular methods do not discriminate between viable and non-viable virions (Mahmoudian et al., 2011) and therefore, test results need to be interpreted carefully, as a positive result may not be indicative of active infection. Identification of serum antibody against ILTV, by viral neutralisation assays or more commonly, by enzyme-linked immunosorbent assay (ELISA) are routinely used to screen for infected/exposed individuals (Bauer et al., 1999; Sander and Thayer, 1997).

This review focuses on current understandings about the innate and adaptive immune responses towards ILTV, as well as the role of ILTV glycoprotein G (gG) in immune evasion strategies. The efficacy of immunity induced by vaccines currently available and under development is also discussed.

2. Innate immune responses towards ILTV infection

Inflammation in response to infection is crucial to pathogenesis by controlling virus replication, contributing to pathologies and directing the subsequent adaptive immune response. The inflammatory response to ILTV infection is critical to both virus virulence and the balance of the adaptive immune response of the host, as demonstrated in studies describing ILTV gG (see below). Lesions associated with ILTV infection can be found in the conjunctiva and throughout the respiratory tract, with lesions most consistently found in the trachea of infected birds (Tripathy, 1998). At this site inflammatory infiltrates significantly impact on the already small tracheal aperture and therefore the respiratory effort of the host. The innate immune responses to ILTV infection are not well understood, although technologies enabling the creation of gene deleted ILTV constructs (Devlin et al., 2006; Fuchs et al., 2005; Mundt et al., 2010; Pavlova et al., 2010; Schnitzlein et al., 1995; Veits et al., 2003) and transcriptional profiling of host cells (Lee et al., 2010, 2012a) are now improving our understanding of events early after infection.

The mucous lining the respiratory tract and the ciliated respiratory epithelium provides a physical barrier for infection at the airway interface. The airway mucous is an innate barrier that virus must penetrate before contact with the respiratory epithelium. This mucous is secreted by intraepithelial goblet cells and is a viscoelastic gel with a structural framework provided by cross-linked mucin glycoproteins (Vareille et al., 2011). This provides a physical barrier to the movement of larger particles, where the diffusion of herpesvirus-sized particles is 100-fold slower through mucous than through water (Cone, 2009). Mucous also contains many innate immune mediators including interferons, collectins, IgA, defensins and lactoferrin (Vareille et al., 2011), although the relevance of these molecules during ILTV infection of the trachea is not known.

The destruction of respiratory epithelium not only disrupts the physical barrier at the airway interface, but infected epithelial cells secrete chemokines and cytokines that direct both the innate inflammatory and the adaptive immune responses (Vareille et al., 2011). Transcriptional profiling of host gene expression using microarray analysis in ILTV infected chicken embryo lung cells infected *in vitro*, show that 789 genes were differentially expressed between infected and uninfected cells. While many differentially expressed genes grouped in functional pathways involved in cellular growth and proliferation and cell death, 54 genes grouped in inflammatory response pathways, in addition to 60 genes in cell signalling pathways (Lee et al., 2010). ILTV infection significantly increased the expression of interleukin-6 (7-fold), interleukin-8 (22-fold) and CXC K60 (19-fold), with much smaller increases in the expression of CCL17 (4-fold), CCL20 (3-fold), CCL4 (4-fold), chemokine ah221 (3-fold), CXCL14 (2-fold) and interleukin-15 (2-fold). While the up regulation of several of these genes during ILTV infection is consistent with the pathogenesis of other herpesviruses, this *in vitro* study analysing the host response of a single cell type is likely to represent only the beginning of the cascade of events expected to occur by a wider range of epithelial and inflammatory cells types *in vivo*.

The extensive hyperplasia of respiratory epithelium observed in histopathology sections of trachea (Purcell, 1971) is consistent with the up regulation of genes related to cell growth and proliferation, while up regulation of cytokine and chemokine genes is consistent with the infiltration of inflammatory cells. Between 0 and 72 h after experimental infection, there is a mild infiltration of small number of lymphocytes and polymorphonuclear cells (PMN, presumably heterophils) in the *lamina propria* in areas where syncytia are present. Between days 3 and 5 post infection, there is more severe oedema of *lamina propria* and underlying tissues, with numerous macrophages and lymphocytes (Purcell, 1971) as well as histiocytes and plasma cells (Guy and García, 2008) throughout the *lamina propria*, and PMNs locating to oedematous areas surrounding capillaries and migration in larger numbers to the hyperplastic epithelium (Purcell, 1971). Immunohistochemistry shows both CD4+ and CD8+ lymphocytes scattered throughout the mucosa, while B lymphocytes formed clusters in the mucosa (Devlin et al., 2010). By this stage of infection, the types of inflammatory cells recruited to the site of infection appear to influence the outcome of infection and the balance of the adaptive response (Devlin et al., 2010). This finding and the variability of different lines of chickens to both susceptibility to ILTV infection and ability to establish a protective immune response (Poulsen et al., 1998), suggests innate immune factors are crucial to the outcome of infection.

3. Adaptive immune responses towards ILTV infection

Viral glycoproteins appear to be most immunogenic antigens, capable of eliciting both humoral and cell mediated responses (York and Fahey, 1990). Cell-mediated immunity, rather than antibody is correlated with protection against disease (Fahey et al., 1983a, 1984; Fahey and York, 1990a; Honda et al., 1994a). An early report by Fahey et al. (1983a,b) demonstrated that circulating antibody was not important in immunity against ILTV. Although passive transfer of antibody to offspring has been demonstrated (Hayles et al., 1976a), no significant differences were found between chicks hatched from hyper-immune and non-vaccinated