Virus Research 185 (2014) 23–31

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

H5N1 infection causes rapid mortality and high cytokine levels in chickens compared to ducks

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ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 6 February 2014
Accepted 11 March 2014
Available online 19 March 2014

Keywords:
H5N1 avian influenza
Cytokine storm
IL6
Chicken
Duck

ABSTRACT

Infection with H5N1 influenza virus is often fatal to poultry with death occurring in hours rather than days. However, whilst chickens may be acutely susceptible, ducks appear to be asymptomatic to H5N1. The mechanisms of disease pathogenesis are not well understood and the variation between different species requires investigation to help explain these species differences. Here we investigated the expression of several key proinflammatory cytokines of chickens and ducks following infection with 2 highly pathogenic H5N1 (A/Muscovy duck/Vietnam/453/2004 (Vt453) and A/Duck/Indramayu/BBVW/109/2006 (Ind109)) and a low-pathogenic H5N3 influenza virus (A/Duck/Victoria/1462/2008 (Vc1462)). H5N1 viruses caused fatal infections in chickens as well as high viral loads and increased production of proinflammatory molecules when compared to ducks. Cytokines, including Interleukin 6 (IL6) and the acute phase protein Serum Amyloid A (SAA), were rapidly induced at 24 h post infection with H5N1. In contrast, low induction of these cytokines appeared in ducks and only at later times during the infection period. These observations support that hypercytokinemia may contribute to pathogenesis in chickens, whilst the lower cytokine response in ducks may be a factor in their apparent resistance to disease and decreased mortality.

1. Introduction

Host–pathogen interactions are critical to the outcome of disease. The initial cellular recognition of pathogens by the pathogen recognition receptors (PRR) triggers downstream immune-related genes and rapidly sets up an overall immune response against the invading pathogen (Wang et al., 2007). Whilst the resultant mileu of cytokines are paramount in the host protection from viruses such as highly pathogenic avian influenza (HPAI), the upregulated cytokines also lead to inflammation at the sites of infection (Sladkova and Kostolansky, 2006; Kobasa et al., 2007). The level of cytokine impact on the disease state is unclear. Infections such as dengue fever (Leong et al., 2007), malaria (Clark et al., 2008) and severe acute respiratory syndrome (SARS) (Cameron et al., 2008) promote an inflammatory cytokine response during early infection which have been suggested to be responsible for cellular and organ damage. H5N1 infections in humans also leads to an increased inflammatory response and appears to contribute to the severity of disease and associated mortality (Tran et al., 2004; Chan et al., 2005; de Jong et al., 2006). The H5N1-associated response includes the regulation of proinflammatory cytokines, antiviral cytokines and interferons (IFNs) (Sladkova and Kostolansky, 2006) which inhibit viral replication (Szretter et al., 2007; Karpala et al., 2008). However, the induction of some of the associated cytokines, like IFN, TNFα, IL1β and IL6, are thought to be at least partially responsible for influenza-induced pathology (Cheung et al., 2002).

Interestingly, the rapid disease progression observed in influenza infected chickens is not observed in ducks (Alexander et al., 1986). Whilst ducks often maintain a healthy state they enable the continuing spread of influenza by acting as a viral reservoir and then mingling with chicken flocks, and so ducks are critical to the control of the influenza cycle (Webster et al., 1992). Research efforts show that humans and other animals, as well as chickens, suffer from high viral loads, extensive tissue tropism, and elevated immune responses following H5N1 infections (Cheung et al., 2002; Suzuki et al., 2009) whereas ducks often remain in good health, all the while continuing to shed virus (Alexander et al., 1986; Jeong et al., 2009). Recent comparative analyses indicates ducks and chickens respond differently to low pathogenic avian influenza

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http://dx.doi.org/10.1016/j.virusres.2014.03.012
0168-1702/

(LPAI) infection (Mundt et al., 2009; Cornelissen et al., 2012). The lungs of the chicken, in contrast to ducks, endure high viral loads as well as high levels of IL6, type 1 IFNs, and PRR such as TLR3, TLR7 and Mda5 (Cornelissen et al., 2012). Nevertheless, studies with mammals and avians show that viral loads and immune responses associated with HP-influenza infections are quite different when compared with LP-influenza infections (Tumpey et al., 2005).

Since the clinical manifestations of influenza-infected chickens and ducks appear to be different, and further, the immune responses to LP-influenza, in contrast to HP-influenza, are distinctly divergent, we investigated the impact of HP-influenza H5N1 infection in chickens and ducks. In this study 2 H5N1 strains, derived from ducks, were used to infect chickens and ducks. The cytokine and acute phase immune responses were analyzed by observing the levels of IFNγ, SAA and the interleukins, IL1β, IL6 and IL18. In addition, the levels of Toll-like receptor 7 (TLR7) were examined as H5N1 viral RNA interacts with TLR7 (Stewart et al., 2012) which further activate innate immune responses (Geeraedts et al., 2008). Vast differences were evident following HP-influenza infections in chickens and ducks. Furthermore, the findings were specific to HP-influenza since LP-influenza H5N3 virus (Vc1462) did not induce similar responses in the chicken. It is hoped that a better understanding of these host–pathogen interactions might be helpful in deriving new immune modulation strategies aimed at reducing HP-influenza induced illness in chickens and humans.

2. Results

2.1. H5N1 influenza has increased severity and mortality in chickens compared to ducks

Two groups of 12 chickens and 15 ducks were inoculated with either Vt453 or Ind109 H5N1 viruses and time to mortality indicated that Vt453-infected chickens succumb to infection most rapidly at 18–24 hours post infection (h.p.i.) (Fig. 1) (3 had to be euthanized due to severe symptoms). Ind109-infected chickens died (or were euthanized) between 24 and 28 h.p.i. The clinical signs associated with Vt453- or Ind109-infected chickens included weakness, depression, ataxia, swelling of the head, labored breathing, fever and seizures. In contrast, Vt453- and Ind109-infected ducks showed none of these clinical signs initially, but between 48 and 72 h.p.i., 3 Vt453-infected ducks showed some depression and weeping eyes. At 72–96 h.p.i. severe disease was observed in 8 of the 15 Vt453-infected ducks, including neurological signs, ataxia and in 4 cases death (or ducks requiring euthanasia). Ind109-infected ducks showed no signs of infection.

2.2. Chickens appear to have rapid and systemic H5N1 viral infection compared to ducks

Tissues were analyzed for virus and the highest viral titers were identified in all H5N1-infected chickens. At 24 h.p.i., viral titers were between 6 and 7 $\log_{10}$ tissue culture infectious doses ($TCID_{50}$) in the lung, spleen and heart whilst the brain contained between 5 and 6 $\log_{10}$ $TCID_{50}$ (Fig. 2). In contrast, ducks had low levels of virus over a 168 h time-course (Fig. 2). Furthermore, ducks differentially responded to the different H5N1 strains. In Vt453-infected ducks, viral titers were 4, 2 and 7 $\log_{10}$ $TCID_{50}$ in the lung, spleen and heart, respectively, at 48 and 72 h.p.i. whilst the Ind109-infected ducks, generally had lower viral titers (Fig. 2). H5N1-infected ducks had between 1 and 2 $\log_{10}$ $TCID_{50}$ of virus in the brain at 72 h.p.i. (Fig. 2).

2.3. H5N1 Vt453 and Ind109 infection increases cytokine and acute phase expression in chickens

Rapid increases in mRNA levels of the proinflammatory cytokines IFNγ, IL1β and IL6 were detected 24 h.p.i., in the spleen, brain, lung and heart of Vt453-infected chickens (Fig. 3). IL6 had the largest mRNA increases (heart 100-fold, spleen 80-fold and lung 70-fold) compared to uninfected controls (Fig. 3). The Ind109-infected chickens also had increased proinflammatory cytokine levels (IFNγ, IL1β and IL6) at 24 h.p.i. although IL6 increases were far less in Ind109-infected chickens compared to Vt453-infected chickens (Fig. 3). Interestingly, the IL6 levels of Vt453-infected chickens were highly elevated compared to the IL6 levels of Vt453-infected duck (Fig. 3). The acute phase protein SAA increased following H5N1-infection in a profile similar to IL6. In the Vt453-infected chickens SAA increased in the heart 80-fold, spleen 40-fold and lung 40-fold – whilst SAA increases in the Ind109-infected chickens were heart 40-fold, brain 10-fold and lung 20-fold (Fig. 3). TLR7 levels were lower in H5N1-infected chickens when compared to the H5N1 infected duck in all tissues at 24 h.p.i.

H5N1 Vt453 and Ind109 infection influences duck inflammatory cytokine levels less than chickens but increases in TLR7 occurs in ducks, In contrast to chickens, both the Vt453- and Ind109-infected ducks had little to no change in IFNγ, IL1β, IL6 and IL18 levels when compared to uninfected controls at 24 h.p.i. There was also no apparent change in the levels of the acute phase gene SAA in duck organs at 24 h.p.i. (Fig. 3). However, duck TLR7 levels were increased in spleen, brain and heart tissue between 2- and 4-fold at 24 h.p.i. At 72 h.p.i. some proinflamatory cytokines increased in the duck spleen, brain and heart which corresponded to peak virus titers at 72 h.p.i. However, in the brain of Vt453-infected ducks IFNγ increased 30- to 40-fold and IL18 5- to 10-fold at 72 h.p.i. (Fig. 4).

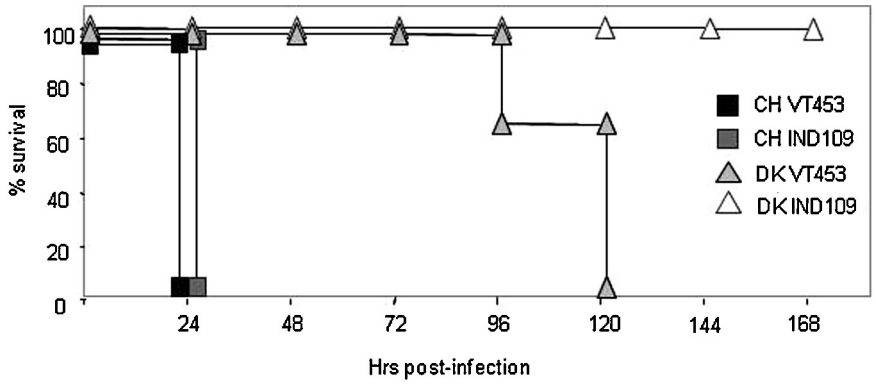

Fig. 1. Survival of chickens following H5N1 infection is decreased in comparison to ducks. Following inoculation with highly pathogenic H5N1 influenza (Vt453 or Ind109) the % survival of chickens (squares) or ducks (triangles) was measured. Chickens succumbed to both viral infections (or had to be euthanized) within 24 h whilst ducks infected with Ind109 appeared asymptomatic. Ducks infected with Vt453 succumbed to infection (or had to be euthanized) at 72–120 h.p.i.