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Association of the chicken MHC B haplotypes with resistance to avian coronavirus

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ABSTRACT

Clinical respiratory illness was compared in five homozygous chicken lines, originating from homozygous B2, B8, B12 and B19, and heterozygous B2/B12 birds after infection with either of two strains of the infectious bronchitis virus (IBV). All chickens used in these studies originated from White Leghorn and Ancona linages. IBV Gray strain infection of MHC homozygous B12 and B19 haplotype chicks resulted in severe respiratory disease compared to chicks with B2/B2 and B5/B5 haplotypes. Demonstrating a dominant B2 phenotype, B2/B12 birds were also more resistant to IBV. Respiratory clinical illness in B8/B8 chicks was severe early after infection, while illness resolved similar to the B5 and B2 homozygous birds. Following M41 strain infection, birds with B2/B2 and B8/B8 haplotypes were again more resistant to clinical illness than B19/B19 birds. Real time RT-PCR indicated that infection was cleared more efficiently in trachea, lungs and kidneys of B2/B2 and B8/B8 birds compared with B19/B19 birds. Furthermore, M41 infected B2/B2 and B8/B8 chicks performed better in terms of body weight gain than B19/B19 chicks. These studies suggest that genetics of B defined haplotypes might be exploited to produce chicks resistant to respiratory pathogens or with more effective immune responses.

1. Introduction

Infectious respiratory diseases are responsible for considerable economic losses in commercial poultry operations. The respiratory avian coronavirus, infectious bronchitis virus (IBV), has consistently been a major agricultural problem in chickens for many years (Cavanagh, 2007; Collisson et al., 1992). IBV was the first coronavirus described (Schalk and Hawn, 1931) and the first for which the entire genome was sequenced (Binns et al., 1985; Boursnell et al., 1987; Collisson et al., 1992). As a non-zoonotic virus, IBV is ideal for characterizing avian host responses to respiratory viruses. Furthermore, it provides a model for the highly contagious severe acute respiratory syndrome (SARS) in humans, which resembles IBV in transmission, pathogenesis and genome structure (Jackwood, 2006).

In chickens, IBV causes a highly contagious acute respiratory disease especially in commercial flocks and is transmitted by aerosol and fecal contamination. While respiratory signs of illness are coughing, sneezing, nasal discharge, gasping and tracheal rales, IBV successfully replicates in various tissues including respiratory, intestinal and reproductive tracts, as well as kidneys and infections have been linked to nephrogenic, enteric and reproductive diseases (Cavanagh and Naqi, 2003; Lucio and Fabricant, 1990; Raj and Jones, 1997). Chickens of all ages can be infected, but morbidity and mortality are generally more severe in very young chicks. Economic losses are mainly attributed to decreased weight gain, feed efficiency, egg production and quality, mortality and condemnation (Cavanagh and Naqi, 2003).

Abbreviations: IBV, infectious bronchitis virus; MHC, major histocompatibility complex; M41, Massachusetts 41; RT-PCR, reverse transcription polymerase chain reaction; SARS, severe acute respiratory syndrome; RSV, Rous sarcoma virus; MDV, Marek's disease virus; SPF, specific pathogen free; PI, post infection; EID_{50}/ml, embryo infectious dose 50 per ml; NIU, Northern Illinois University; PBS, phosphate buffer saline; RNA, ribonucleic acid; 5′ UTR, 5′ untranslated region; bp, base pairs; ANOVA, analysis of variance; ARK, Arkansas; CTL, cytotoxic T lymphocyte; AIV, avian influenza virus; IFNγ, interferon gamma; poly I:C, polyinosinic polycytidylic acid; USDA, United States Department of Agriculture; NIFA, National Institute of Food and Agriculture.

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IBV infection induces humoral and cellular immune responses in chickens, which result in viral clearance and protection against challenge infection (Cavanagh, 2007; Collisson et al., 2000; Ignjatovic and Galli, 1994; Pei et al., 2001; Raj and Jones, 1997; Seo et al., 2000). The first IBV vaccine strains, which have undergone many *in vivo* and *in vitro* passages, were derived from the classical IBV Massachusetts 41 (M41) and the closely related Beaudette strains (Cavanagh and Naqi, 2003; Hodgson et al., 2004). M41, a prototype strain of IBV, was first isolated in 1941 by Van Roekel at the University of Massachusetts (Fabricant, 1998; Jungherr et al., 1956). During the following decades, numerous serologically and genetically distinct strains were identified throughout the world. Molecular studies suggest that continuing evolution of the viral spike protein, the target protein of neutralization, can result in vaccine breaks and IBV outbreaks (Bochkov et al., 2006; Cavanagh and Naqi, 2003; Ignjatovic and Sapats, 2000; Ignjatovic and Galli, 1994). Variations are a result of the common occurrences of point mutations and recombination events (Cavanagh et al., 2005; Jackwood et al., 2012; Wang et al., 1994, 1993).

The severity of infection with IBV may depend, in part, on the genetics of the infected chickens, including differences in immune responses (Bacon et al., 2004; Cook et al., 1990; Nakamura et al., 1991; Otsuki et al., 1990; Zekarias et al., 2002). Successful selection and breeding of chickens for disease resistance is a rational approach to reducing disease-related losses. Inbred White Leghorn line 15I birds were shown to be more susceptible to IBV challenge than line C, which exhibited more severe clinical symptoms and a greater amount of virus was recovered from the respiratory tract and kidneys (Cook et al., 1990; Nakamura et al., 1991; Otsuki et al., 1990). A retrospective study implicated the MHC B region in resistance to IBV where the vaccinated chicks with B13 and B21 haplotypes experienced higher mortalities than those with the B15 haplotype (Bacon et al., 2004). The B15 haplotype has been associated with Rous sarcoma virus (RSV) progression of tumors and sensitivity to Marek's disease virus (MDV), and the B2 haplotype was associated with RSV regression and the B21 with moderate regression of RSV induced tumors (Bacon et al., 2000).

The chicken B complex, which includes the B–F or MHC class I genes, B–L or MHC class II genes and B–G genes, unique to birds (Miller et al., 1988; Plachy et al., 1992), consists of at least 242 genes, mostly related to innate and adaptive immune responses (Briles and Briles, 1987; Shiina et al., 2007). There are over 50 genetically defined chicken MHC or B haplotypes (Fulton et al., 2006). Although the MHC B region has been implicated in resistance, a clinically controlled study examining the extent of susceptibility and resistance to IBV induced disease in a spectrum of B haplotypes has not been reported.

The current studies compare the clinical respiratory illness associated with the pneumotropic and nephrotropic IBV Gray strain infection of chickens from five homozygous lines and one heterozygous line defined by their MHC B haplotypes. Birds with B haplotypes selected on the basis of their Gray strain associated respiratory disease were infected with a second IBV strain, the pneumotropic M41, for scoring of clinical illness, viral load and performance.

2. Materials and methods

2.1. Viral stocks

The pneumopathogenic M41 and the moderately pneumopathogenic and moderately nephropathogenic Gray strains of IBV were separately propagated in 10-day-old specific pathogen free (SPF) chicken embryos (Sneed et al., 1989). The morbidity (any respiratory illness during any of the days examined) resulting from infection with either strain was >90% in these studies. The IBV M41 strain was further passaged twice in 4–5-day-old SPF chicks. The virus was collected from the pooled supernatants of trachea and lungs when respiratory signs were observed in chicks at 6 and 8 days post infection (PI) for passage 1 and at days 7, 9, 11 and 13 days PI for passage 2. The viral titers of the IBV strains were determined in embryonated chicken eggs by the Reed and Muench method (1938) and expressed as embryo infectious dose 50 per ml (EID_{50}/ml). The viral stocks were stored at −80 °C.

2.2. Experimental animals

All chicks used in these experiments were hatched and housed in an SPF environment. Eggs with embryos, that were homozygous for B19, B2, B8, B5 and B12 haplotypes and the heterozygous B2/B12 haplotype, were obtained from Northern Illinois University (NIU), DeKalb, IL (Briles and Briles, 1982). The NIU chicken lines, with simultaneous segregation of nine alloantigen genes and particular MHC genes, were derived from unvaccinated chickens in flocks negative for IBV and avian influenza and have been maintained as closed flocks at NIU since 1970. Two chicken stocks of diverse origins (White Leghorn and Wisconsin inbred line 3 Ancona breeds) have been selected for crosses to yield full and half-sib families (Briles, 2004). All matings were designed to utilize parents from multiple families of the desired B haplotype with minimal inbreeding, where full and half sib matings were avoided. All NIU eggs were obtained from birds, whose MHC B haplotypes had been identified and were distinguishable by serology and microsatellite typing. All the homozygous B haplotype eggs were collected from several matings, in which all parents were homozygous for the 19, 12, 2, 5 or 8 B type allele (Briles and Briles, 1982; Fulton et al., 2006). The heterozygous B2/B12 chicks were obtained from several matings of B2/B2 dams and B12/B12 sires. The SPF eggs used for the titration of virus were obtained from Avian Vaccine Services (Charles River Laboratories, Inc., North Franklin, CT). All birds were humanely euthanized by isoflurane overdose. Animal experiments were approved by the institutional animal care and use committees of Texas A&M University and Western University of Health Sciences.

2.3. Experimental design

Each experimental group was housed separately in an SPF environment with free access to feed and water. Chicks were inoculated by the oculo-nasal route at 5–6 days of age with either the Gray or M41 strain of IBV or with buffered phosphate (PBS) as controls (Pei et al., 2001; Seo and Collisson, 1997). In the initial IBV Gray experiment, 24 birds in each experimental group (B19/B19, B12/B12, B2/B2, B5/B5 and B2/B12) were infected with 10^6 EID_{50} of Gray strain per bird, while the infected B8/B8 group had 36 birds and all uninfected control groups had 12 birds per group. In a separate second experiment, using B haplotype defined birds infected with $2 \times 10^{1.3}$ EID_{50} IBV M41, 13, 17 and 21 birds were used in the uninfected control B2/B2, B19/B19 and B8/B8 groups, respectively, while 16, 20 and 21 chicks were used in the infected B2/B2, B19/B19 and B8/B8 groups, respectively. In a separate experiment quantifying M41 *in vivo*, seven 6-day-old SPF chicks were used for inoculation of each 10-fold (10^{-1} and 10^{-5} in PBS) dilution of M41 ($10^{3.3}$ EID_{50}/ml), while 12 age-matched uninfected control chicks were inoculated with an equivalent volume of PBS. The chicks given M41 were inoculated with 0.2 ml and those given Gray were inoculated with 0.1 ml total volume of virus per chick in PBS. The IBV Gray study was conducted in the Lab Animal Research Resources facility at Texas A&M University, College Station, TX; while the IBV M41 studies were performed in the Animal