Veterinary Immunology and Immunopathology 140 (2011) 30–36

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Pathogenic and immunogenic responses in turkeys following *in ovo* exposure to avian metapneumovirus subtype C

Ra Mi Cha[1], Mahesh Khatri[2], Manohar Mutnal[3], Jagdev M. Sharma*

Department of Veterinary and Biomedical Sciences, University of Minnesota, 1971 Commonwealth Ave., St. Paul, MN 55108, United States

ARTICLE INFO

Article history:
Received 15 May 2010
Received in revised form 2 November 2010
Accepted 10 November 2010

Keywords:
Avian metapneumovirus
Turkeys
In ovo vaccine

ABSTRACT

Commercial turkey eggs, free of antibodies to avian metapneumovirus subtype C (aMPV/C), were inoculated with aMPV/C at embryonation day (ED) 24. There was no detectable effect of virus inoculation on the hatchability of eggs. At 4 days post inoculation (DPI) (the day of hatch (ED 28)) and 9 DPI (5 days after hatch), virus replication was detected by quantitative RT-PCR in the turbinate, trachea and lung but not in the thymus or spleen. Mild histological lesions characterized by lymphoid cell infiltration were evident in the turbinate mucosa. Virus exposure inhibited the mitogenic response of splenocytes and thymocytes and upregulated gene expression of IFN-γ and IL-10 in the turbinate tissue. Turkeys hatching from virus-exposed eggs had aMPV/C-specific IgG in the serum and the lachrymal fluid. At 3 week of age, *in ovo* immunized turkeys were protected against a challenge with pathogenic aMPV/C.

Published by Elsevier B.V.

1. Introduction

In ovo vaccination, first reported in 1982 with Marek's disease (Sharma and Burmester, 1982) has been used to protect chickens against a number of viral infections (Ahmad and Sharma, 1992; Sharma, 1985; Sharma et al., 2002; Stone et al., 1997; Wakenell et al., 1995). In this procedure, live viral agents are inoculated into the amniotic sac of late-stage embryos. Multiple viral agents may be combined in one injection and induce protection against all the components in the mixture (Sharma et al., 2002). The viral agents inoculated into eggs replicate in a variety of embryonal tissues although the host–viral interactions vary among viruses (Ahmad and Sharma, 1992; Corley and Giambrone, 2002; Corley et al., 2002; Guo et al., 2004; Hess et al., 2004; Khatri and Sharma, 2009; Rautenschlein and Haase, 2005; St Hill and Sharma, 2000; St Hill et al., 2004; Worthington et al., 2003; Zhang and Sharma, 2003). *In ovo* vaccination in chickens against Marek's disease virus alone or in combination with other agents such as infectious bursal disease virus has become an industry standard in the U.S. and a number of other countries (Schijns et al., 2008)

Avian metapneumovirus (aMPV) is an important naturally occurring pathogen that causes upper respiratory tract (URT) infection and immunosuppression in turkeys. There are three principal subtypes of the virus: A, B and C. Subtypes A (aMPV/A) and B (aMPV/B) are known to be prevalent in Europe and other countries whereas subtype C (aMPV/C) has been detected in the U.S. (Seal, 2000; Shin et al., 2002) and recently in South Korea (Lee et al., 2007). There are major differences in the host range and pathogenesis of aMPV/C in comparison with aMPV/A and aMPV/B. aMPV/C causes natural infection in turkeys but not in chickens; aMPV/A and aMPV/B infect both chickens and turkeys (Buys et al., 1989; Cook et al., 1993; Cook, 2005). Although

* Corresponding author. Present address: The Biodesign Institute, Arizona State University, 1001 S. McAllister Ave., Tempe, AZ 85287, United States. Tel.: +1 480 727 6066; fax: +1 480 727 0599.
E-mail address: jagdev.sharma@asu.edu (J.M. Sharma).
[1] Present address: Southeast Poultry Research Laboratory, USDA, Agricultural Research Service, 934 College station Rd., Athens, GA 30605, United States.
[2] Present address: Department of Food Animal Health Research Program, Ohio Agricultural Research and Development Center, Ohio State University, 1680 Madison Ave., Wooster, OH 44691, United States.
[3] Present address: Department of Medicine, Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, 2001 6th St SE Minneapolis, MN 55455, United States.

0165-2427/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetimm.2010.11.006

lesions induced by all three subtypes remain localized in the URT, aMPV/A and aMPV/B but not aMPV/C cause substantial damage to the trachea (Catelli et al., 1998; Chary et al., 2002a; Cook et al., 1991; Cook, 2005; Jirjis et al., 2000, 2002; Majo et al., 1995; Panigrahy et al., 2000).

Currently, aMPV/C in commercial turkey flocks in U.S. is controlled by vaccination with a live attenuated vaccine (Patnayak and Goyal, 2004). This vaccine is administered to the hatched turkeys in the drinking water. Our objective was to examine the possibility of immunizing commercial turkeys with aMPV/C by administrating the live virus into eggs at ED24. We examined the embryos and newly hatched turkeys for (a) virus replication in embryonic tissues; (b) presence of lesions in the URT; (c) the onset and the duration of aMPV/C-specific IgG in the serum and the lachrymal fluid; (d) cytokine gene expression in the URT; and (e) *in vitro* mitogenic proliferation of splenocyte and thymocyte. In addition, we assessed the protective ability of *in ovo* immunization against pathogenic virus challenge. Our results indicate that the embryos responded vigorously to the virus and developed protective immunity.

2. Materials and methods

2.1. Virus

The avian metapenumovirus/Minnesota/turkey/1a/1997 isolate of aMPV/C at the 41st passage in Vero cells was obtained from Dr. S. Goyal, University of Minnesota (Goyal et al., 2000) and was used for immunization as well as challenge. This virus, although attenuated, has residual pathogenecity and causes extensive respiratory lesions in turkeys (Cha et al., 2007). At ED 24, 5×10^3 $TCID_{50}$ of the virus were inoculated into each turkey egg via the amniotic route as described previously (Ahmad and Sharma, 1992; Hess et al., 2004; Schijns et al., 2008; Sharma et al., 2002; Worthington et al., 2003). For the challenge, 5×10^3 $TCID_{50}$ of aMPV/C were given oculonasally (O/N) to each turkey.

2.2. Turkeys

aMPV/C antibody-free Nicolas strain commercial turkey eggs were obtained from Jenny-O-Turkey Store (Barron, WI). Serum from 1-day-old turkeys was examined by enzyme-linked immunosorbent assay (ELISA) (Chiang et al., 2000) to ensure absence of anti-aMPV/C antibodies. Turkey eggs were incubated in a clean and disinfected egg incubator and after hatch, each treatment group was maintained in separate Horsfall isolators under the regulations of the University of Minnesota Animal Care and Use Committee. All experimental procedures related to turkeys were also approved by this Committee.

2.3. Enzyme-linked immunosorbent assay (ELISA)

An aMPV/C-specific IgG ELISA was performed as described previously with modifications (Chiang et al., 2000). Serum was obtained from the blood samples collected from the wing vein of the turkeys and the lachrymal fluid was collected by sodium chloride stimulation as described previously (Cha et al., 2007; Ganapathy et al., 2005). The test sera were diluted 1:40 and the lachrymal fluid samples were diluted 1:10 in a dilution/blocking buffer (KPL, Gaithersburg, MD) and 50 μl of sample per well was added to a 96-well plate coated with aMPV/C antigen or control antigen (Vero cell extract). After 1 h, HRP-conjugated goat anti-turkey IgG antibody (KPL, Gaithersburg, MD) was added as a secondary antibody. Tetramethylbenzidine (TMB) was used as a developing reagent. The plates were washed 3 times with 0.05% Tween-20 containing PBS between each step. All reactions except TMB treatment were done at 37 °C and the TMB treatment was conducted at room temperature. Virus-specific IgA ELISA was performed as described previously (Cha et al., 2007).

2.4. Histopathology

Paraffin-embedded sections of the turbinate tissue (4 μm) were stained with hematoxylin and eosin (H&E). Each tissue section was examined at 100× magnification in a blind manner and was given a lesion score of 0–4 depending upon the severity of lymphocyte infiltration in the mucosal layer (0 = less than 20% of the mucosal layer showing lymphoid cell infiltration; 1 = 20–40%; 2 = 40–60%; 3 = 60–80%; 4 = 80–100%.). Because normal turkeys have lymphoid aggregates in respiratory mucosa, the MLS of virus-exposed group was normalized with the mean MLS of untreated controls. Turkeys lack lymph nodes and lymphoid aggregates in normal birds are considered mammalian lymph node equivalents.

2.5. Quantitative RT-PCR (qRT-PCR)

qRT-PCR was developed to examine gene expression of IFN-γ, IL-10, IL-18 and aMPV/C. The three cytokines were selected because they represent Th1 and Th2 immune responses and turkey sequences are available in the Genbank. For the cytokine genes, total RNA was extracted from the turbinate tissue using TRIzol regent (Invitrogen, Carlsbad, CA). For the viral genes, the Viral RNA Extraction Kit (Qiagen, Valencia, CA) was used to extract viral RNA from choanal swabs. cDNA synthesis was performed with equal amounts of RNA using Superscript III Reverse transcriptase (Invitrogen, Carlsbad, CA). qRT-PCR for turkey cytokines and aMPV/C M protein were developed based on the protocols for chicken cytokine genes (Hong et al., 2006; Kaiser et al., 2000; Kaiser et al., 2003; Palmquist et al., 2006) and the published protocols for aMPV/C qRT-PCR (Munir et al., 2006; Velayudhan et al., 2005). Turkey GAPDH was used as an internal control to normalize turkey gene expression. The primers for turkey IL-10, IL-18, GAPDH and aMPV/C were designed by Primer 3 (http://jura.wi.mit.edu/rozen/papers/rozen-and-skaletsky-2000-primer3.pdf) based on respective gene sequence from Genbank. Primers for IFN-γ were constructed from chicken IFN-γ gene and it showed 100% homology with turkey IFN-γ gene (Kaiser et al., 2000). Primer sequences used for qRT-PCR are shown in Table 1. PCR was conducted using Brilliant® II QPCR SYBR® Green QPCR Master Mix (Stratagene, La Jolla, CA) and amplification and detection was performed in an automated