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Newcastle disease virus vectored infectious laryngotracheitis vaccines protect commercial broiler chickens in the presence of maternally derived antibodies

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ABSTRACT

Newcastle disease virus (NDV) recombinants expressing the infectious laryngotracheitis virus (ILTV) glycoproteins B and D have previously been demonstrated to confer complete clinical protection against virulent ILTV and NDV challenges in naive chickens. We extended this study to assess whether maternally derived antibody (MDA) against NDV and ILTV would interfere with protection in vaccinated broiler chickens. Chickens with a mean NDV MDA hemagglutination inhibition (HI) titer of 6.4 (log_2) and detectable ILTV neutralization (VN) antibodies at hatch were vaccinated with rLS/ILTV-gB or rLS/ILTV-gD at 1 or 10 day of age (DOA) or on both days. Groups of birds vaccinated with the commercial ILT vaccines (FP-LT and CEO) or sham inoculated were also included in this study. All vaccinated birds were challenged with virulent ILTV strain at 21 DOA. By that time, NDV HI titers declined to 2.6 (log_2) in unvaccinated birds, whereas the HI titers in NDV vectored vaccine groups increased to 3.5–6.3 (log_2). At standard dosages, both vaccine candidates conferred significant clinical protection; however, the protection elicited by the rLS/ILTV-gD was superior to that of rLS/ILTV-gB. Recombinant rLS/ILTV-gD reduced ILTV shedding from tracheal and ocular tissues by approximately 3 log_{10} $TCID_{50}$. Notably, there was no improvement in protection after booster vaccination at 10 DOA. Overall results indicate that the presence of maternal antibodies to NDV and ILTV did not significantly interfere with the ability of the NDV LaSota strain-vectored ILTV gB and gD vaccine candidates to elicit protective immunity against infectious laryngotracheitis.

1. Introduction

Infectious laryngotracheitis (ILT) is an acute upper respiratory tract disease of chickens caused by the alphaherpesvirus infectious laryngotracheitis virus (ILTV) or gallid herpesvirus type 1 (GaHV-1). Despite the extensive use of attenuated, and more recently recombinant vaccines, ILT continues to affect poultry on a worldwide basis [1]. Historically, the commonly used live ILT vaccines were first attenuated in the 1950s by either multiple passages in embryonated eggs (chicken embryo origin [CEO]) or in tissue culture (tissue culture origin [TCO]) [2,3]. Although these vaccines protect against clinical disease, they have residual virulence, which is exacerbated by continued infections of naïve birds from productively infected animals and latent carriers [4–6]. In high-density poultry rearing facilities there is a continuous reservoir of ILT viruses, both virulent and vaccinal. It has been reported that CEO vaccinal "revertants" can become the dominant field strains in poultry populations and cause outbreaks [7,8]. Although the recombinant vaccines, turkey herpesvirus (HVT) and fowlpox virus (FPV) expressing ILTV antigens are safe [9–14], they only induce partial protection when compared with that induced by live-attenuated vaccines [15]. Thus, a significant need exists to revise the ILT control strategies particularly regarding the

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development of next generation vaccines that are safe, protective and incapable of vaccinal reversion.

Recently, we developed Newcastle disease virus (NDV) recombinants expressing the ILTV glycoproteins B and D as bivalent vaccines [16]. Vaccination of one-day-old specific pathogen free (SPF) and 3-week-old commercial boiler chickens conferred complete clinical protection against virulent ILTV and NDV challenges [16]. However, there was a general concern that the maternally derived antibodies (MDA) may interfere with the immunoresponses of the chicks to the corresponding vaccines [17–19]. It has been reported that the NDV MDA confers protection to young chicks against ND, but it can also interfere with NDV vaccine efficacy at 1 day of age (DOA) [17,19]. This interference is dependent on the titer of maternal antibodies and the strain of the NDV vaccine administered [20,21]. The LaSota vaccine strain has been demonstrated to overcome MDA interference better than the Hitchner, B1 or V4 strains [21]. Higher levels of circulating maternal antibodies to the LaSota strain were required to depress the immunoresponse than that required to do the same for the other three strains [21]. In contrast, maternal antibodies to ILTV do not confer protection against disease nor interfere with the immune response following vaccination [22].

To evaluate whether maternal antibodies interfere with protection induced by the LaSota strain-vectored ILTV recombinants, rLS/ILTV-gB and rLS/ILTV-gD, we carried out vaccination/challenge experiments with one-day-old commercial broiler chickens that had a mean NDV MDA HI titer of 6.4 ($\log_2$) and detectable ILTV virus neutralization (VN) antibodies. The immunoresponses following vaccination and protection indices (e.g. clinical signs, body weight gain and virus shedding after ILTV challenge) suggest significant protection against ILTV challenge even in the presence of maternally derived antibodies specific for NDV and ILTV.

2. Materials and methods

2.1. Vaccines, viruses and cells

The generation of NDV LaSota strain-vectored ILTV vaccine candidates, rLS/ILTV-gD and rLS/ILTV-gB, were previously described [16] and propagated in 9-day-old SPF chicken embryos at passage level 3. The titers of these vaccine stocks were determined by the 50% egg infective dose (EID_{50}) assay in 9-day-old SPF chicken embryos [23]. The commercial ILTV vaccines, Vectormune (FP-LT) and Trachivax (CEO), were purchased from Ceva Biomune (Lenexa, KS) and Merck Animal Health (Summit, NJ), respectively. The ILTV strain (63140/C/08/BR) was obtained from the pathogen repository bank at the Poultry Diagnostic and Research Center (PDRC, University of Georgia, Athens, GA) and used as the challenge virus [11,24]. A recombinant ILTV expressing the green fluorescent protein (ILTV/GFP) was used to infect LMH cell line (ATCC® CRL-2117™) in order to measure neutralizing maternal antibody titers against ILTV [25]. LMH cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Life Technologies, Carlsbad, CA) and antibiotics (100 U/ml Penicillin, 100 μg/ml Streptomycin, 0.25 μg/ml Amphotericin B, Thermo Scientific, Suwanee, GA) at 37 °C in 5% CO_2 atmospheres. The titers of the ILTV challenge virus and the ILTV/GFP virus stocks were determined by the 50% tissue infectious dose ($TCID_{50}$) assay in primary chicken kidney (CK) cells in a 96-well format [11,23].

2.2. Animals

One-day-old commercial broiler chickens were obtained from Fieldale Farms (Baldwin, GA). The breeders of these commercial broiler chickens were vaccinated with ILT vaccine (TCO) once and with NDV vaccines (B_1B_1 and LaSota strains) multiple times before layering eggs. Newly hitched birds were transferred to the BLS-2E animal facilities at the Southeast Poultry Research Laboratory (SEPRL). Birds were housed in Horsfal isolators (Federal Designs, Inc., Comer, GA) with *ad libitum* access to feed and water. At the termination of the experiments all birds were humanely euthanized in accordance to an SEPRL's Institutional Animal Care and Use Committee approved animal use protocol.

2.3. Animal experimental design

One hundred and fifty one-day-old commercial broiler chickens were randomly divided into ten groups of 15 birds. Each bird in groups 1 and 2 was inoculated with 100 μl of phosphate buffered saline (PBS) via intranasal (IN) and intraocular (IO) routes and served as unvaccinated controls. Birds in groups 3, 4, and 5 were vaccinated with 100 μl of rLS/ILTV-gD (1.0×10^7 EID_{50}/ml) per bird via IN/IO routes at 1 (group 3) or 10 (group 4) DOA or both days (group 5). Birds in groups 6, 7, and 8 were vaccinated with 100 μl of rLS/ILTV-gB (1.0×10^7 EID_{50}/ml) per bird via IN/IO routes at 1 (group 6) or 10 (group 7) DOA or both days (group 8). Birds in groups 9 and 10 were vaccinated with one dose of commercial vaccines via the subcutaneous (Vectormune FP-LT at 1 DOA) or eye drop (CEO at 10 DOA) routes as instructed by the manufacturers (Ceva and Merck, respectively). At 21 DOA, the birds in groups 2–10 were challenged with the virulent ILTV (strain 63140/C/08/BR) at a dose of 10^4 $TCID_{50}$/per bird via intra-tracheal route. Birds in group 1 were untreated and served as non-challenged controls for clinical sign observations and body weight gains. After challenge, clinical signs of ILT were observed and recorded daily for ten days as previously described [16,26]. Briefly, breathing patterns, conjunctivitis, and the level of depression were scored on a scale of 0 to 3: normal (0), mild (1), moderate (2) and severe (3). A score of 9 was given to a bird that died. The total clinical sign scores per group per day were calculated. Blood samples were collected prior to both vaccination (1 DOA and 10 DOA) and challenge (21 DOA) for determining MDA titers and immunoresponses. Body weights were measured prior to challenge and at 10 days post challenge (DPC). Tracheal and ocular swabs were collected from each bird at 4, 7 and 10 days post-challenge (DPC) and stored in Brain Heart Infusion (BHI) medium (Becton, Dickinson and Company, Sparks, MD) at −80 °C until further needed.

2.4. Quantitation of ILTV load in tracheal and ocular swabs

The ILTV viral load in each tracheal and ocular swab sample was quantified using a quantitative real-time PCR assay (qPCR) with the host α2-collagen gene as an internal control [16,27]. DNAs were isolated from the swab samples using a DNeasy 96 Blood & tissue kit (Qiagen). The amount of ILTV DNA was measured by the qPCR assay on an Applied Biosystems® 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA) with specific primers and probes as described previously [16]. A threshold value of 0.05 was used for consistent evaluation of different 96-well plates. The amount of challenge infectious laryngotracheitis virus shed in tracheal and ocular samples was calculated by two methods: standard curve and the relative $2^{-\Delta\Delta CT}$ method. A series of 10-fold dilutions of titered ILTV challenge virus stock was subjected to the DNA isolation and the qPCR assay in order to establish a standard curve correlating ILTV titers with copy numbers of viral DNA. Samples with any recorded threshold cycle number (Ct) value were considered positive and used to approximate ILTV titers. Based on the qPCR Ct values of the samples, the ILTV titers were converted by referencing the standard curve of ILTV titers/qPCR Ct values. The mean titers of ILTV from each of the treatment groups were expressed in Log_{10} plus standard deviation (SD). The second

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