Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

Early immune responses and profiling of cell-mediated immunity-associated gene expression in response to rHVT-IBD vaccination

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ARTICLE INFO

Article history:
Received 4 September 2017
Received in revised form 8 December 2017
Accepted 19 December 2017
Available online xxxx

Keywords:
Recombinant vaccine
Turkey herpesvirus
$CD8^+$ cell-mediated immunity
Infectious bursal disease
Chicken

ABSTRACT

Infectious bursal disease (IBD) remains a major threat to the poultry industry. Recombinant herpesvirus of turkey (rHVT)-IBD vaccines have been successfully used to induce a protective immune response against IBD. However, the capacity for rHVT-IBD vaccines to induce early protection without detectable antibodies, and the underlying mechanisms mediating specific cell-mediated responses in the early stages following vaccination, have been poorly investigated. Therefore, in this study, specific pathogen-free (SPF) chickens were vaccinated with rHVT-IBD and T-cell subsets were analyzed. Both splenic and circulating $CD8^+$ cell populations increased at 7 days postvaccination (dpv). Next, the expression of adaptive immunity-related genes was analyzed in the spleen and lung of rHVT-IBD-vaccinated chickens. Upregulation of CD8 expression was observed at 7 dpv. Interestingly, a parallel increase in the transcription of granzymes A and K was also detected from 7 dpv. To our knowledge, the latter result is the first to be reported, and it suggests that cytotoxic activity of $CD8^+$ T lymphocytes is activated. In contrast, expression of the innate genes examined remained largely unchanged following vaccination. To further investigate the IBD virus (IBDV)-specific responses triggered by rHVT-IBD vaccination, vaccinated chickens were inoculated with an attenuated IBDV strain with the aim of restimulating induced immune responses *in vivo*. The expression profiles of various genes associated with adaptive immune responses were subsequently analyzed in lung, spleen, and bursa of Fabricius samples. Significant upregulation of CD4, CD8, perforin, and IFNγ expression were observed in the bursa samples 7 days postinoculation (dpi). In the lung, transcript levels of CD8, granzymes and perforin were also significantly higher in the rHVT-IBD-vaccinated chickens at 7 dpi, thereby suggesting that specific cellular immune responses were activated. Overall, these results support the hypothesis that stimulation of specific $CD8^+$ cell-mediated immunity contributes to the response against IBDV in rHVT-IBD-vaccinated chickens.

1. Introduction

Herpesvirus of turkey (HVT) is a *Mardivirus* that belongs to the *Alphaherpesvirinae* subfamily, along with the pathogenic Marek's disease virus (MDV). In field trials, HVT strain FC126 was identified as a good candidate vaccine against MDV based on its efficacy [1]. HVT was also identified as an efficient vector for the delivery of foreign antigens. Consequently, HVT has been used to create recombinant vaccines against several avian pathogens [2–5].

Infectious bursal disease (IBD) is a highly contagious disease and a global threat to the poultry industry. IBD virus (IBDV) targets immature B lymphocytes in lymphoid organs [6] and targets the bursa of Fabricius of young chickens, thereby causing death, disease, or immunosuppression and leading to secondary infections with opportunistic pathogens and vaccination failures [7]. However, recombinant HVT (rHVT)-IBD has been successfully used to protect chickens against IBDV [8–10] by expressing the capsid protein VP2 of IBDV [2]. Moreover, unlike live attenuated IBDV vaccine strains that retain some residual pathogenicity, rHVT-IBD is not associated with a risk of transient immunosuppression [8].

Despite rHVT vaccines being efficacious against a variety of avian pathogens, the immune mechanisms related to the protection elicited by these vaccines have not been entirely elucidated. In chickens vaccinated with rHVT, persistent viremia is established and promotes a long-lasting antibody response that can be measured between 3 weeks postvaccination (wpv) and 30 wpv [11,12]. However, recent studies highlighted good clinical protection against IBDV challenges in the early stages following rHVT-IBD vaccination, despite the detection of low levels of anti-VP2 antibodies, thereby indicating the contribution of other arms of the immune responses [13]. We previously reported the ability of

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https://doi.org/10.1016/j.vaccine.2017.12.059
0264-410X/

rHVT-IBD vaccines to induce cell-mediated immunity at 3 wpv in specific pathogen-free (SPF) chickens based on an analysis of interferon-gamma (IFNγ) production in response to *ex vivo* recall stimulation of splenocytes with a live IBDV strain [14]. Moreover, rHVT-IBD vaccines were demonstrated to induce some cross-protection against IBDV heterologous strains [13]. When rHVT vaccines were used to immunize against avian influenza (AI), this approach was also successful in eliciting protection against a heterologous challenge, despite low hemagglutination inhibition (HI) titers. Furthermore, a cell-mediated cytotoxic immune response was also demonstrated *in vitro* against various subtypes of AI virus with rHVT vaccines [15].

After inoculation of 18 day-old embryos, HVT was shown to initially replicate in the lung before it spreads to peripheral organs, including the spleen, thymus, and bursa [16–18]. In the present study, distinct T cell subsets were first profiled in splenic and peripheral blood lymphocytes populations early after SPF chickens were vaccinated with rHVT-IBD. In addition, the expression of innate and adaptive immunity genes in the lung and the spleen were examined. To further investigate the IBDV-specific responses triggered by rHVT-IBD vaccination, an *in vivo* approach involving vaccination with rHVT-IBD followed by an inoculation with an attenuated IBDV was performed with the goal of specifically re-stimulating immune responses in vaccinated chickens.

2. Materials and methods

2.1. Chickens

SPF White Leghorn chickens embryonated eggs were obtained from Lohmann Valo (Cuxhaven, Germany). After hatching, all birds were kept in biosecurity level 3 (BSL-3) isolators, with access to food and water provided *ad libitum* throughout the experimental period. Animal experiments were conducted with authorization and supervision from the Biosafety and Bioethics Committees at the Veterinary and Agrochemical Research Institute (Brussels, Belgium) according to national and European regulations.

2.2. Vaccine and virus

The rHVT-IBD Vectormune® vaccine was provided by Ceva Santé Animale (Lenexa, KS, USA). Classical IBDV strain D78 was purchased from MSD Animal Health (Milton Keynes, UK).

2.3. Experimental design

Protocols for the two animal experiments conducted in this study are summarized in Table 1.

Experiment 1: This experiment was conducted to assess: (i) potential changes in splenic and circulating immune cell subsets by flow cytometry; and (ii) the expression of various genes in spleen and lung of vaccinated chickens. Thus, chickens were immunized at 14 days of age with a subcutaneous injection in the neck of an inoculum of ten commercial doses (3.6 log10 plaque-forming units (pfu)/dose) of vaccine in 100 µl of corresponding vaccine diluent (Ceva-Biomune, KS, USA). Three animals per group were humanely sacrificed at 2, 5, 7 and 9 days postvaccination (dpv). Spleen, lung, and blood samples were collected at each time point.

Experiment 2: This experiment was conducted after Experiment 1 in order to evaluate the expression of several genes related to adaptive immunity in the lung, spleen, and bursa of vaccinated chickens following *in vivo* recall stimulation with an attenuated strain of IBDV. The experimental design was similar

Table 1
Experimental design and summarized protocol of Experiment 1 and Experiment 2.

Animal experiment	Group (chickens/group)	rHVT-IBD vaccination			IBDV inoculation				Sampling timings (chickens/timing)	Samples
		Age at vaccination (days)	Vaccine dose (titer)	Inoculation route (volume)	Age at inoculation (days)	Viral titer	Inoculation route (volume)			
1	Unvaccinated (12)	–	–	–	–	–	–		2, 5, 7, 9 days postvaccination (3)	Spleen, lung, blood
	rHVT-IBD-vaccinated (12)	14	10 commercial doses (3.6 log10 pfu/dose)	Subcutaneous (in 100 µl)	–	–	–			
2	Unvaccinated (9)	–	–	–	–	–	–		2, 5, 7 days postinoculation with IBDV (3)	Spleen, lung, bursa
	rHVT-IBD/- (9)	14	10 commercial doses (3.6 log10 pfu/dose)	Subcutaneous (in 100 µl)	–	–	–			
	rHVT-IBD/IBDV (9)	14	10 commercial doses (3.6 log10 pfu/dose)	Subcutaneous (in 100 µl)	21	10^4 TCID50	Oculo-nasal (in 100 µl)			
	Unvaccinated/IBDV (9)	–	–	–	21	10^4 TCID50	Oculo-nasal (in 100 µl)			