

IMMUNE-PROTECTION MECHANISMS AGAINST LATE CHALLENGE WITH AVIAN INFECTIOUS BRONCHITIS VIRUS (IBV) INDUCED BY A SINGLE-DOSE OF AN ATTENUATED VACCINE IN DAY-OLD CHICKS

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ABSTRACT

The essential function of existing vaccines against primary respiratory pathogens such as IBV is to elicit, ideally, local specific antibodies, as well as cell-mediated immunity (CMI) against this virus. To study the mechanisms of protective immunity, the levels of local specific antibodies and the expression of genes associated to cytotoxic T cell activity, such as CD8-marker and granzyme-A on tracheal mucosa were evaluated after the primary immunization of 1-day-old chicks with an attenuated Massachusetts IBV vaccine and challenge 41 days later. Protection was evaluated 5 days post-infection (dpi) with virulent IBV strain (M41) by tracheal ciliostasis analysis, histopathology and virus detection by real time RT-PCR. The lachrymal anti-IBV antibody levels of IgG, IgM and IgA isotypes were measured by an ELISA method. The expression of CMI genes were evaluated by real time RT-PCR at 1, 5 and 10 dpi. The results showed that 42-day old chickens vaccinated at 1-day old were protected against challenge with the homologous virulent IBV strain. Early increases after challenge of lachrymal IgG, IgM and IgA anti-IBV specific antibodies and in the levels of expression of CMI genes in the tracheal mucosa are associated with effective protection of respiratory tract against IBV infection, characterized by less viral detection, inflammation and tracheal damage. Overall, the development of humoral and CMI memory responses against IBV induced after early vaccination at the primary viral replication site are involved in conferring relevant protection mechanisms against viral infection and could be assessed for monitoring or the development of novel IBV vaccines.

KEY-WORDS: vaccine immunity, immunoglobulin isotypes, cellular immunity, AND tracheal mucosa.

INTRODUCTION

Infections of the respiratory tract, such as that caused by infectious bronchitis virus (IBV) are among those capable of inducing significant economical impact on poultry production worldwide, by causing damages on respiratory, and/or uro-genital tracts of breeders, layers and broilers (Cavanagh, 2007). Vaccination strategies for IBV,

despite their relative success in routine use, have yet some drawbacks. Thus, more vaccination studies in chickens are needed, specially those related to the immune responses elicited after vaccination, that provided the main effector mechanisms to protect against this viral infection. An efficacious and long-lasting protection against IBV infection requires the activation of both effector and memory cell-mediated immunity (CMI) and humoral immunity (HI) against this virus (Cavanagh, 2007).

During the course of viral infections, particularly IBV infection, CMI responses mediated by systemic CD8 T cells and a number of mediators produced by these cells, such as Granzyme A, Granzyme B and perforins, have shown relevant roles in the destruction of viral infected cells (COLLISON et al., 2000, GÖBEL et al., 2001, WANG et al., 2006).

However, currently immunological methods to detect and measure CMI and mucosal antibody immune responses in chicken are scarce and need to be extended to generated useful and relevant data. The availability for reliable bio-assays and ELISAs to be applied in evaluation of anti-IBV post-vaccination immunity is even more limited, though molecular methods, such transcriptome and quantitative RT-PCR technique were developed and applied to evaluate gene expression at tracheal site after IBV infection (WANG et al, 2006). Therefore, the development of molecular methods, such as quantitative RT-PCR for measuring CMI responses at respiratory tract along with the quantification of IgA, IgG and IgM anti-IBV antibodies in respiratory tract secretions, would be helpful for a better understanding of the immune responses induced by attenuated IBV vaccines. These approaches can be used to estimate the correlation of these immune responses with the protection status in the respiratory tract against an experimental infection with a virulent homologous strain of IBV. In this study, birds immunized with a commercial attenuated vaccine against IBV and challenged after six weeks, were used to check out whether CMI and HI anti-viral responses could be measured at tracheal sites in order to correlate these immune responses with the effective protection against this virus.

MATERIAL AND METHODS

Experimental design: Four groups of 15 SPF (Specific Pathogen Free) chicks (Leghorn Lineage) were used. The birds were housed in positive pressure isolators. Groups I, II and received, respectively, full dose (10^4 DIE₅₀, as recommended by the manufacturer), a sub-dose ($10^{3.5}$ DIE₅₀) of attenuated H120 vaccine strain of IBV at the first day of age, by ocular-nasal route. Group III received only the vaccine diluent and group IV remained unvaccinated. At 42 days of age, groups I, II, and III were experimentally infected by intra-ocular and intranasal routes with $10^{5.0}$ EID₅₀/bird IBV (M41 strain). Lachrymal secretion samples were collected after 1, 4, 7, 14, 21, 28, 35, 42, 43, 47 e 52 days post-vaccination (dpv), and also at 1, 5, and 10 days post-infection (dpi), and stored at -20°C. Five chickens from each group were sacrificed at 1, 5 and 10 dpi. Tracheal samples were collected from each group, a portion immediately frozen and kept at -70°C until processing, and the remaining was subjected to histopathological and ciliostasis analysis.

Measurement of local antibodies against IBV: Local (tears) IgG, IgA and IgM were measured from samples collected from the birds of the experimental groups by Sandwich-ELISA-Concanavalina A (S-ELISA-ConA) as recommended by BRONZONI et al. (2005).

Histopathology and Ciliostasis analysis: Samples of tracheas were processed and evaluated for histopathological and ciliary movement analysis as described by ANDRADE et al. (1992).

RNA extraction and reverse transcription: The extracted RNA from tracheal samples was treated with DNase I and reverse transcribed by standard procedures.

Viral replication: A pair of primers flanking HRV2 region at S1 gene of IBV was used to measure the level of viral replication on tracheal samples of chickens infected with IBV, by Real-time using with SYBR Green I marker (OKINO et al., 2006)

Relative quantification of expression of genes related to cell-mediated immunity: The relative quantification of gene expression of CD8+ marker of cytotoxic cells, and Granzyme A on tracheal samples was performed by Real-time PCR using SYBR Green I. Gene expression was normalized related to GAPDH gene, using the birds of group IV (non vaccinated and non infected) as basal gene expression levels (WANG et al., 2006).

Statistical analysis: Differences between the groups of experimental design were analyzed by Kruskal-Wallis test followed by Dunn's, with $p < 0,05$. Correlation analysis between the tests were determined by Spearman, with $p < 0,05$.

RESULTS AND DISCUSSION

Local Specific Antibody Isotypes Induced by an Vaccination with an attenuated IBV strain: a slight and significant increase in IgG and IgM anti-IBV antibodies was detected in tear samples from day-old vaccinated chickens, between 5 to 6 weeks post-vaccination, which was followed by an early and more pronounced increase at 1, 5 dpi. The IgA anti-IBV antibodies raise only after challenge in these birds. Conversely, the non-vaccinated birds only show significant IgM, IgG and IgA anti-IBV responses at 10 dpi. MONDAL and NAQI (2001) have demonstrated the involvement of local specific antibodies in the control of IBV infection, but no correlation studies between memory of local HI with anti-IBV protection to challenge have been reported so far.

Expression of genes related to CMI: the full-dose vaccinated chickens showed an early and significant increased expression of CD8 gene (2 fold) at 1dpi, which lasted at this level up to 5 dpi, increasing 10 fold at 10 dpi. The level of CD8 gene expression raised in sub-dose vaccinated birds at 5 dpi (2 fold) and at 10 dpi (10 fold). Conversely the expression of CD8 gene delayed in non-vaccinated birds, and increased significantly, 0.8 to 35 fold at 5 and 10 dpi, respectively.

The Granzyme A showed a similar kinetic profile, increasing early for full-dose vaccinated birds (2 fold, at 1 dpi), and maintained high levels at 5 and 10 dpi (8 fold). The sub-dose vaccinated group and non-vaccinated birds, raised later their levels of the expression of Granzyme A (6 to 8 fold, at 5 dpi), and or increased moderately (8 fold) or markedly (38 fold) their expression levels at 10 dpi. Specific cytotoxic T lymphocytes have been shown to be crucial for the systemic clearance of this virus impairing more pronounced kidney lesions induced by a nephropathogenic IBV strain (COLLISON et al, 2000) and here we find a CMI memory response (early rise in CMI genes) against IBV in trachea, which is the primary infectivity site for this virus.

Tracheal pathological changes in vaccination-challenge test : experimental vaccination of one-day-old chicks with a full-dose of attenuated H120 IBV strain provided late protection to challenge (42 day of age) of tracheal epithelial surface, since all immune birds conserved, after challenge with M41 virus, high levels of ciliary

activity and showed significant reduced tracheal lesions; In addition, the virus was detected from the trachea in only one out of five birds by real time RT-PCR. The sub-dose vaccinated birds showed similar tracheal damages in terms of ciliary activity and histopathology, but the IBV was detected in the trachea of two out of five birds the IBV, by real time RT-PCR. On the contrary, the control group of non-vaccinated birds showed a marked ciliostasis in the epithelial cells along with prominent tracheal lesions, and the virus was yielded from the tracheal samples of all five challenged birds.

CONCLUSION

We have developed and applied reliable quantitative RT-PCR and ELISA assays to measure CMI and HI anti-IBV responses at respiratory tract of chickens. Additionally, the overall results of this study lead us to assume that local antibodies and cellular immune mechanisms, specially the memory CMI and HI responses are elicited after vaccination with commercial attenuated Massachusetts IBV vaccine, and may act in an additive or synergistic way, in order to confer a more complete protection status against IBV infection at the primary infective site.

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