In vitro evaluation of live attenuated vaccines against Salmonella enteritidis: cell-mediated immune response

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ABSTRACT

The use of Salmonella enteritidis (SE) live attenuated vaccines is one of the major tool to reduce this infection in commercial poultry. In this work, techniques, evaluating the presence and the expression of some cytokines, were studied to improve the knowledge of the cellular-mediated immune response following SE vaccination. This study demonstrated that SE vaccination enhances the production of INF-γ, IL-8, iNOs, while downregulates IL-1β. Between these immunologic parameters, the evaluation of INF-γ seems to be the most significant and easy test to plan and optimize SE vaccination programs.

Key words: Salmonella enteritidis, Live vaccine, Cellular-mediated immune response.

RIASSUNTO

VALUTAZIONE IN VITRO DI UN VACCINO VIVO ATTENUATO CONTRO SALMONELLA ENTERITIDIS: RISPOSTA IMMUNITARIA CELULLO-MEDIATA

Una delle maggiori strategie nel controllo di Salmonella enteritidis (SE) è l’uso della vaccinazione con ceppi vivi attenuati. Per poter ottimizzare l’utilizzo dei vaccini vivi, in questo studio sono state messe a punto alcune tecniche di valutazione della risposta immunitaria cellulare mediata. È stato dimostrato come la vaccinazione induca l’aumento di INF-γ, IL-8, INOS, mentre IL-1β è down-regolata. Tra tutti questi parametri la determinazione di INF-γ sembra essere quello più significativo e applicabile per l’ottimizzazione dei piani vaccinali.

Parole chiave: Salmonella enteritidis, Vaccino vivo, Risposta immunitaria cellulare mediata.
Introduction

Host acquired immunity to *Salmonella* spp. includes T-cell mediated and B-cell mediated responses which are important to protect the host from the pathogens (Lillehoj *et al.*, 2003). The cell-mediated immunity acts in the early phases of the infection when the humoral immune response is still lacking. The aim of this study was to evaluate the efficacy and protection of a live commercial SE vaccine checking the cell-mediated immune response.

Material and methods

Animals and immunization

Twenty 1 day-old commercial laying hens were housed in a cage and eyedrop vaccinated at 14 and 28 days following the label instructions (Gallivac SE, Merial, France). Ten birds were reared separately as negative controls.

Mitogen and antigen induced lymphocyte proliferation

Splenic cells from fresh spleens were isolated as described in Okamura *et al.* (2004). The cells were seeded in tissue culture plates and stimulated with Concanavaline A and with antigen specific SE, produced as described in Beal *et al.* (2004). After 24 hours the supernatants were collected and analyzed for the presence of INF-γ, IL-1β, IL-8 and iNOs mRNA.

ELISA for INF-γ

INF-γ in cell culture supernatants was detected with commercial ELISA Kit "Chicken INF-γ CytoSets" (Biosource) as described by the supplier.

mRNA extraction and Real time PCR

mRNA was extracted with RNAeasy Mini Kit (Qiagen) with two DNase digestions as described by the supplier.

cDNA was obtained with specific primers for IL-8, IL-1β, iNOs and β-actin, as reference gene, with Real time PCR (Sadeyen *et al.*, 2005). Standard DNA plasmids were used to quantify IL-1β, IL-8 and iNOs. The results are showed as average values in Figures 1, 2 and 3.

Results and discussion

Five vaccinated and three not vaccinated birds were sacrificed one week post vaccination and the spleens were collected. After splenocytes stimulation, cells culture supernatant was collected and analyzed to quantify INF-γ, IL-1β, IL-8 and iNOs. The results are showed as average values in Figures 1, 2 and 3. INF-γ is a major Th1 cytokine which plays an important role in protection against *Salmonella* infection in avian host through the activation of macrophages to produce NO (Lillehoj *et al.*, 2003). INF-γ production by splenocytes stimulation was quantified by ELISA. Spleen cells of vaccinated birds showed higher INF-γ value than unvaccinated birds when were stimulated with ConA and SE. SE response was weaker than ConA; the vaccine induced a strong general cell-mediated response.

IL-1β is a pro-inflammatory cytokine produced by macrophages and it is involved in the mediation of the acute phase in inflammatory response following challenge (Lillehoj *et al.*, 2003). The IL-1β level was higher in not vaccinated birds than in the vaccinated ones. This down-regulation was also described by Kaiser (2000) after *Salmonella* infection leading to a reduced rapid inflammatory response in the intestine allowing the initial entry of bacteria. IL-8 is a chemokine induced likely by invasion of *S. enteritidis* and it enhances heterophil influx in the chicken intestine (Kaiser *et al.*, 2000). Like with INF-γ, vaccination increased IL-8 production in the spleen cells stimulated with ConA and SE antigen and this increment was higher with ConA. Okamura *et al.* (2003) also detected the IL-8 increment in serum 7 days post vaccination. iNOs is the protein involved in the NO synthesis that is generally produced by host macrophages.
Figure 1. INF-γ quantity in vaccinated birds (black columns) and not vaccinated (white columns) one week post vaccination with stimulation by ConA and SE antigen.

![ELISA INF](chart)

Figure 2. IL-1β quantity in birds vaccinated (black columns) and not vaccinated (white columns) one week post vaccination after stimulation with ConA and SE antigen.

![IL-1β](chart)

in response to invading Salmonella. NO is a potent oxidant with bactericidal effects. Vaccination stimulated iNOs production. As previously described by Lillehoj et al. (2003) iNOs production was induced by INF-γ.

**Conclusions**

In Salmonella infection, it has been known that the cell-mediated immunity is the only one protective in the early phases when the humoral response is absent. Moreover it is effective in the Salmonella intracellular phase.

We have observed that SE live vaccine administration enhances INF-γ, IL-8 and NO. The increment of these immune effectors indicates that the host response are clearly augmented by the live vaccine. INF-γ is a reliable parameter to evaluate the cell-mediated immune response after vaccination. INF-γ
ELISA is a rapid and easy technique to perform without expensive equipment. Because Salmonella live vaccine programs are influenced by the immunity status, somministration route and infection pressure, the use of INF-γ information could be important to optimize the use of these vaccines.

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