Andrzej Puchalski

Synthetic C-type oligodeoxynucleotides with non-methylated CpG sequences as a component increasing the immunogenicity of a commercial calf vaccine containing the IROMP *Mannheimia haemolytica* antigen

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Bovine Respiratory Disease Complex (BRDC) is one of the most important health problems in calf rearing. Difficulties in implementing an effective immunoprophylaxis of this disease result mainly from low efficacy of vaccine preparations available on the market. Increased effectiveness of available vaccines can be achieved using carefully selected adjuvants. Synthetic oligodeoxynucleotides containing non-methylated CpG sequences mimicking bacterial DNA could be used as potential adjuvants for enhancing primarily the cellular branch of the immune response towards vaccine antigens. The available literature lacks reports on the possibility of using CpG ODN as a vaccine adjuvant in preparations for cattle. Few publications relate mainly to vaccines with experimental antigens or antigens of intracellular pathogens, in which B-type CpG ODN was usually conjugated with other adjuvants.

In our own research, C-type CpG ODNs were used as an adjuvant component in a vaccine for cattle, which is an innovative element of the activities undertaken. The aim of the study was (1) to determine the immunostimulatory effects of C-type CpG ODNs in relation to bovine PBMC in *in vitro* tests and compare them to the results obtained by other classes of synthetic oligodeoxynucleotides, for which such activity has already been confirmed, (2) to trace the dynamics of shaping the humoral and cellular immunity after administration of a developed biopreparation to calves in order to compare it with a commercial vaccine, (3) to assess the safety level of using the C-type CpG ODN as an additive for calf vaccines.

In vitro studies on bovine PBMC evaluated the ability of CpG ODN to induce the proliferation of these cells and the secretion of IFN-γ. Cytometric studies determined the cell types that were the most susceptible to immunostimulatory properties of CpG ODN. The Real-Time PCR reactions assessed the gene expression for characteristic cytokines in the Th1-dependent and Th2-dependent responses and genes for IP-10 and TLR9. After confirming the immunostimulatory properties of C-type CpG ODN in relation to bovine PBMC, the essential research step was initiated, which consisted in administering 0.5 mg CpG ODN to the calves together with a commercial vaccine containing IROMP from M. haemolytica as antigen. *In vivo* studies assessed serum levels of IROMP-specific IgG, IgG1, IgG2 and IgM antibodies up to the 70th day after the first vaccination. IP-10 levels in the sera of vaccinated calves were also determined. During the experiment, through the daily measurement of internal body temperature and determination of the serum haptoglobin level, the acute phase response was evaluated in the vaccinated calves.

C-type CpG ODN was found to be the most effective in *in vitro* stimulation of bovine PBMC proliferation, and the main proliferative cell type in response to this ODN was lymphocyte B. C-type CpG ODN was the most effective inducer of IFN-γ production in bovine PBMC cultures at a dose of 8 μg/ml. The obtained mean IFN-γ concentration values in the culture supernatants were, however, statistically significantly lower ($P < 0.05$) than those obtained in the case of A-type CpG ODN. In Real-Time PCR reactions, all tested genes in bovine PBMC were amplified due to their stipulation by C-type CpG ODN applied in the study at a dose of 8 μg/ml.

The addition of C-type CpG ODN to the commercial vaccine formulation positively influenced the increase of its immunogenicity, as evidenced by high levels of serum IROMP-specific IgG, IgG1, IgG2 and IgM antibodies. Both specific humoral-type response (increase in IgG1 and IgM antibodies) and the lymphocyte Th1-mediated cellular-type response were significantly improved, as evidenced by high levels of IgG2 antibodies and induction of IFN-γ production. It has been shown for the first time that IP-10 may be a reliable biomarker of elevated IFN-γ production at cattle, in response to CpG ODN. Clinical studies have shown that C-type CpG ODNs are relatively safe for calves.

Based on the results of presented studies, it can be concluded that various immunological C-type CpG ODN initiated effects confirm their ability to act as vaccine adjuvants inducing faster, stronger and more long-lasting mechanisms of a specific immune response to *M. haemolytica* IROMP. Obtained results may contribute to the development of a vaccine formulation allowing to induce both humoral and cellular immune response against vaccine antigen. In practice, this should reduce the calf's morbidity to respiratory tract diseases.