Selected parameters of immune response in pregnant sows vaccinated against *Trueperella pyogenes*

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Abstract

Expression of CD4, CD8, and CD25 surface markers on T lymphocytes and levels of IFNγ, IL-10, and TNF-α in colostrum and milk were determined in sows vaccinated against *Trueperella pyogenes* in the final stage of pregnancy. The autovaccine, prepared from *Trueperella pyogenes*, administered twice to pregnant sows six and three weeks before the anticipated delivery significantly increased the percentages of TCD4+, TCD8+, and TCD25+ as well as levels of IFNγ, TNF-α, and IL-10 in colostrum and milk. The enhanced immune potential of colostrum effectively protected the piglets against *T. pyogenes* infections during weaning and thus reduced the economic losses on the particular farm concerned, where *T. pyogenes* infections occur endemically. Knowledge of the profile of cellular and humoral immune response in colostrum and milk of vaccinated sows will enable the design of a *T. pyogenes* infection prophylactic programme for suckling pigs and weaners, which are most susceptible to infections.

Key words: sows, *Trueperella pyogenes*, autovaccine, immune response, milk, colostrum.

Introduction

Impaired mechanisms of immunity are responsible for the success of opportunistic microorganisms and hence for increasingly high morbidity and mortality of pigs exposed to them. The classic example is *Trueperella pyogenes* residing on the skin, the mucous membranes of the alimentary or upper respiratory tract and the genitourinary system in swine. Under favourable conditions, *T. pyogenes* brings ailments leading to high economic losses in pig farming. Amongst numerous factors causing *T. pyogenes*-associated diseases in pigs, functional immune disorders, especially the immunocompromising aspect of coexisting infections, e.g. PRRSV, SHV-1, and *M. hyopneumoniae*, are fundamental (1, 4, 5).

The major factor of *T. pyogenes* virulence is intracellular pyolysin, the toxin exerting cytolytic, dermonecrotic, and lethal effects on numerous types of cells, including neutrophils and macrophages. Moreover, pyolysin activates the complement system, stimulates the production of cytokines, and inhibits oxygen burst and bactericidal activities of neutrophils and monocytes (1, 6). *T. pyogenes* is also able to adhere to the alimentary and respiratory epithelial mucosa of a host. The main role in this adhesion is played by NanH and NanP neuroaminidases, which also increase mucous viscosity. This viscosity favours the colonisation of tissues and impairs the immune response of the host by the exposure of IgA to bacterial proteases (3, 4). The other factors of *T. pyogenes* virulence include serine proteases, the calcium-dependent proteins of proteolytic activity. They are involved in invasion and destruction of tissues. *T. pyogenes* escapes from the host immune control and modulation of the immune system during infection and inflammation (3, 6). A wide array of *T. pyogenes* virulence factors results in various disease symptoms,
such as subcutaneous, intramuscular, and internal organ abscesses, and inflammatory lesions in the lungs, joints, mammary glands, and central nervous system (1, 4, 6).

The method of choice to prevent economic losses and to treat T. pyogenes infections is antibiotic therapy. However, its efficacy is low as in most cases the diseases recur with the appearance of antibiotic-resistant strains. Therefore, pig farming must meet the challenge of finding novel ways of prevention and treatment of T. pyogenes infections (11). Since the commercial biological preparations are lacking, autovaccines should be used for immunoprophylaxis.

The objective of the study was to assess the immune responses in sows vaccinated against T. pyogenes infection during the final stage of pregnancy. This assessment was through analysis of CD4⁺, CD8⁺, and CD25⁺ expression on T lymphocytes and levels of IFNγ, IL-10, and TNF-α in colostrum and milk.

Material and Methods

Animals. The study was conducted in the closed cycle pig farm with 50 sows, aged 2-4 years and weighing 150-200 kg. The farm was equipped with a delivery room with six delivery boxes and four pavilions designed for weaners and fatteners, in which the rule of "all-in-all-out" was followed. Pregnant feeding sows were fed individually-appropriate to the pregnancy stage and number of piglets, whereas weaners and fatteners received full portions of fodder ad libitum in groups. From mating to the 100th gestational day sows were kept in single pigsties on the litter. Two weeks before parturition, the sows were transferred to a delivery room with a concrete floor lined with litter. The zoosanitary conditions for sows with piglets as well as for weaners and fatteners were considered good. The farm was free of porcine reproductive and respiratory syndrome (PRRS), pleuropneumonia, and mycoplasmosis, which was confirmed by screening tests carried out in the reference diagnostic laboratory (National Veterinary Research Institute in Pulawy, Poland). In the farm, pigs were prophylactically vaccinated against colibacteriosis, parvovirosis, and erysipelas.

Endemic infections with T. pyogenes have been observed on the farm for several years, which was confirmed by bacteriological tests. The clinical symptoms indicating T. pyogenes infections had already developed in the final period of piglets’ suckling phase from their sows; however, their highest incidence and main infliction of losses were observed during the final stage of fattening. Depending on the animals category, viz. suckling piglets, weaners, fatteners, or sows, clinical symptoms were noted in 5% to 20% of individuals. The suckling piglets, weaners, and fatteners mainly developed multi-articular inflammations of the limbs manifested as peri- and intra-articular abscesses involving the adjacent soft tissues and as limping. Additionally, some percentage of fatteners examined after slaughter showed numerous deep abscesses located in the liver, pleura, peritoneum, mesentery, and muscles. In sows, on the other hand, purulent foci were mainly located in the mammary gland complexes, occasionally involving also the limb joints.

Autovaccine preparation. An autovaccine was prepared from a T. pyogenes strain isolated from sows on the study farm with clinical symptoms of infection. The isolated colonies of T. pyogenes were cultured on Columbia Agar and incubated in 5% CO₂ and at 37°C for 24 h. The T. pyogenes colonies were collected from the plate surfaces; their density was determined using the McFarland scale, and the bacterial culture was inactivated with the phenol solution in the amount suitable to reach a final concentration of 0.3%. The density of bacterial suspension in the vaccine before inactivation was 6 × 10⁸ cells/mL. Inactivation was carried out at 72°C for 3 h. The autovaccine sterility was controlled by culturing on the media for aerobic and anaerobic bacteria and fungi. The safety of the autovaccine was tested on white mice, which received 0.2 mL of the preparation intraperitoneally. After vaccination, the mice were observed for 48 h.

Experimental animals and experimental design. Twelve sows in the final gestational period were used in the study, divided into two equal groups. Group I was administered the autovaccine intramuscularly in a dose of 3 mL/sow, twice at 21-day interval. The first dose was given six weeks before the anticipated delivery (the beginning of colostogenesis) and the booster dose three weeks later, i.e. three weeks before delivery. Group II (the control) was not injected with the autovaccine. All the pregnant and post-parturition sows were under clinical observation. The cellular and humoral immune indices were determined in colostrum and milk immediately after delivery and at 24, 48, 72, 96, 120, 144, and 168 post delivery.

Sampling and preparation of cell suspension in colostrum and milk. The colostrum (5-10 mL) and milk (5 mL) were collected by manual milking from each sow, eight times at 1, 24, 48, 72, 96, 120, 144, and 168 h after parturition, using oxytocin when necessary. The secretion from several glands was collected into sterile vials and processed immediately as per Pomorska-Möl et al. (9). The Second Local Ethics Committee in Lublin approved all the procedures involved in the study.

Samples of mammary secretions were centrifuged at 3 000 rpm for 30 min. The fat upper layer and supernatant were discarded. The colostral cell pellets, containing the cellular part of mammary secretions, were resuspended in 1 mL of media (RPMI-1640 with 10% Foetal Bovine Serum (FBS) and antibiotic/antimycotic) and washed twice by centrifugation (1 500 rpm for 10 min).
Evaluation of the expression of CD4, CD8, and CD25 cell surface markers on lymphocyte surfaces. The studies were conducted by flow cytometry (Epics XL Beckman - Coulter, Comesa CH-Werfen Company, USA) using single labelling CD4 and CD8 with monoclonal antibodies against CD4 and CD8 surface molecules present on swine T lymphocytes. To evaluate the expression of CD25 receptor for IL-2, mouse monoclonal antibodies against CD25 present on activated T lymphocytes were used during the first stage of labelling and the rabbit FITC-labelled antibody against mouse IgG1 was used during the second stage (Serotec Immunological Excellence, U.K.).

Determination of IFNγ, TNFα, and IL-10 levels in colostrum and milk. The levels of cytokines in colostrum and milk were determined using porcine INF-γ ELISA, porcine TNF-α ELISA, and porcine IL-10 ELISA kits (R&D Systems, USA).

Statistical analysis. The data were analysed using the Student t-test and STATISTICA ver.6.1 program.

Results

In group I, the percentage of T lymphocytes with CD4+ phenotype ranged from 33.1% to 45.25% and was significantly higher compared to group II (24.6%–30.4%), except for results at 72 and 144 h after delivery, which were not statistically significant (Fig. 1).

Likewise, the percentage of cells with CD8+ phenotype in vaccinated sows was found to be significantly higher at all measurement points, except for the 168th post-parturition hour where the results were not significant (Fig. 2).

Significant differences were also observed in mean percentages of CD25-containing T lymphocytes between group I and group II, except for 48 and 96 h, when the differences were not significant (Fig. 3).

Moreover, the levels of cytokines were different in the experimental and control groups. The concentration of IFNγ in colostrum and milk of vaccinated sows (group I) was significantly higher in all measurement points compared to the controls (group II) (Fig. 4).

Likewise, the levels of IL-10 were significantly higher in group I in all measurement points except for 96 and 120 h after delivery (Fig. 5).
The levels of TNF-α in both groups showed different tendencies (Fig. 6). During the first 48 h after delivery, high levels of TNF-α were observed only in group I; while its level was almost undetectable in group II. Increased levels of TNF in this group were not noted before 72 h and increased until the 96th h after delivery to significantly higher values than those in group I. At the remaining measurement points, the mean levels of TNF-α in group II were lower compared to vaccinated sows. In vaccinated pigs, the level of TNF exhibited the tendency to decrease gradually with time after delivery. In contrast, the highest level of TNF was observed in non-vaccinated pigs 72 and 96 h after delivery.

Discussion

The mammary gland in sows is a complex of highly specialised tissues involved in supplying the newborn piglets with suitable amounts of energy and nutrients necessary to survive. The colostrum also provides maternal antibodies formed after antigen stimulation of the sow immune system, as their epitheliocchorial placenta enables the transfer of immunoglobulins to foetuses (2, 5, 7, 10, 12). Moreover, piglets are not able to respond actively to antigens, especially those colonising the mucosa of the alimentary tract and respiratory system. The protection against these pathogens is provided by immune cells supplied by colostrum and milk (lactogenic immunity) (5, 8, 10, 12). The methods used in the study enabled the assessment of the profiles of immune response in pregnant sows after double administration of inactivated autovaccine against T. pyogenes infections.

The administration of the autovaccine initiated the humoral and cellular responses, which were evidenced by significantly increased percentages of TCD4+, TCD8+, and TCD25+, and higher levels of IFNγ, TNF-α, and IL-10 in colostrum and milk, compared to the non-vaccinated group. Noteworthy was that the highest percentage of cells bearing CD molecules was found at the same measurement points as the highest levels of cytokines. The autovaccine had no suppressive effects on immunocompetent cells, thus did not reduce the reactivity of the immune system in pregnant sows. In group I significantly higher percentages of TCD4+, TCD8+, and TCD25+, as well as increased levels of IFNγ, TNF-α, and IL-10 compared to controls, indicate an enhanced immunological potential of colostrum, which is the main pathway of transmission to suckling piglets of specific and non-specific passive immunity and the factor protecting against asymptomatic infections in sows’ mammary gland complexes during this period (5, 10).

In the immune response to infection or to an antigen contained in the vaccine, the auxiliary/inductive TCD4+ and suppressive/cytotoxic TCD8+ lymphocytes are the regulatory cells and their proportion determines the immune response profile. A significant increase in the percentage of CD25+ containing T lymphocytes was observed in the colostrum and milk of vaccinated sows. The CD25 molecule is expressed on activated T lymphocytes, particularly on Th1 lymphocytes, and it is the receptor of IL-2 (10, 12). Thus, it can be concluded that significantly increased percentages of T lymphocyte subpopulations and higher levels of cytokines in the colostrum and milk of vaccinated sows provided efficient protection to piglets against T. pyogenes infections and raised their survival rates. Moreover, the lack of clinical symptoms in piglets from vaccinated sows during weaning reduced the economic losses on the farm where T. pyogenes infections occurred endemicly.

In our studies, the profile of immune response in sows after administration of the autovaccine was also determined by assessing the colostrum and milk levels of IFN-γ, IL-10, and TNF-α, which are the cytokines acting antagonistically. IL-10, produced mainly by Th2 lymphocytes, plays a key role in immune response regulation by reducing the reactivity of the immune system in newborns, supporting the humoral response, and inhibiting the production of cytokines by Th1
lymphocytes. Moreover, after passing to the intestines of suckling piglets, IL-10 contained in sow colostrum and milk, together with TGF-β1 and IL-6, increases production of intestinal IgA antibodies and works for the maturation of their immune system (10). The concentrations of TNF-α in both groups of sows were slightly different. In the vaccinated group, the highest mean values of TNF-α were observed during the first 48 h after delivery whereas in the control group the level of this cytokine at that time was undetectable. It cannot be excluded that increased TNF-α levels in the colostrum of vaccinated sows during the first post-parturition days are associated with its important regulatory role in inflammatory and immunological processes (5, 10). Together with IL-2 and IL-6, TNF-α markedly increases proliferation and differentiation of lymphocytes as well as synthesis and release of other cytokines. These interactions determine the protective mechanisms of the host that are essential to limit the course of T. pyogenes infections and develop specific antibacterial immunity.

In conclusion, vaccination of pregnant sows with a T. pyogenes-based autovaccine increases the immunological potential of colostrum and milk. Knowledge of the profile of cellular and humoral immune responses in colostrum and milk of vaccinated sows will help to develop the protection programme against T. pyogenes infections in suckling piglets and weaners, which are the most susceptible to infections.

References