

## INTRADERMAL AND INTRAMUSCULAR PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS) VACCINATION IN PIGLETS: CHANGES OF PERIPHERAL LYMPHOCYTE SUBPOPULATIONS.

P. Borghetti<sup>1</sup>, E. De Angelis<sup>1</sup>, F. Miduri<sup>1</sup>, S. Gozio<sup>2</sup>, A. Blanchaert<sup>2</sup>, L.G. Alborali<sup>3</sup>, P. Cordioli<sup>3</sup>, G. Lombardi<sup>3</sup>, C. Quintavalla<sup>1</sup>, S. Guazzetti<sup>4</sup>, P. Martelli<sup>1</sup>

<sup>1</sup>Dept. of Animal Health - University of Parma - Italy, <sup>2</sup>Intervet Italia - Milan-Italy, <sup>3</sup>IZS Brescia; Italy, <sup>4</sup>Azienda USL- Reggio Emilia-, Italy

Key words: PRRS, lymphocyte subpopulations, intradermal and intramuscular vaccination

### Introduction

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) causes reproductive failure in sows and respiratory disorders in pigs frequently associated with multiple infections (viruses, *Mycoplasma* and bacteria).

Several experiments have been performed to investigate the humoral and cellular immune response during PRRSV infection in pig (1, 5, 6, 7, 8, 9).

In spite of the observation that the exposure to the virus induces a protective immunity against re-exposure to the homologous virus, it has been also demonstrated that infected pigs developed a prolonged viremia and a persistent infection in lymphoid tissue and that they can become reinfected to the heterologous virus. These evidences testify the variable features of PRRS immune response and the consistent discrepancy between the experimental and field observations about the efficiency of the immune response, such as the supposed immunosuppressive role of PRRSV or the possible vaccine failure (2).

Experimental and field results confirmed that the interaction between the pig immune system and PRRS virus is complex and that the current knowledge on innate and adaptive immune response is almost incomplete.

This paper aims at providing further contribution to the study of PRRSV immunity by the investigation into the peripheral lymphocyte subpopulations by flow cytometric analysis as markers after PRRS vaccine administration by differential routes.

### Materials and methods

Thirty 25-28 day-old piglets were randomly selected from a PRRSV positive-stable 300 sows herd. Animals in growing and fattening phases were PRRSV free. At the time of inclusion piglets have been divided in 3 groups (10 pigs each) and isolated in appropriate locations.

One week later 10 piglets have been vaccinated with a PRRS live vaccine (Porcilis PRRS – Intervet International BV) intramuscularly (group 1) and 10 piglets have been vaccinated via intradermal route using the I.D.A.L.<sup>®</sup> syringe. Group 3 (10 animals) has been kept unvaccinated as negative control.

On a weekly basis all the animals of the three different groups have been bled.

For the characterization of lymphocyte subpopulations, 50  $\mu$ l of heparinized blood were mixed with 5  $\mu$ l of the specific antibodies in a plastic tube.

The used antibodies were: Mouse anti-pig CD4a-R-PE, 74-12-4; Mouse anti-pig CD8a-FITC, 76-2-11. All antibodies were purchased from Valter Occhiena (Turin, Italy).

After 15 min of incubation in the dark at room temperature, the cells are washed with PBS/1%FCS and centrifuged for 5 min at 400 xg. The contaminating red cells are lysed by treatment with NH<sub>4</sub>Cl solution, pH 7,2, for 15 min at room temperature in the dark. The cell suspension is then washed twice with PBS/1%FCS, centrifuged for 5 min at 400 xg, re-suspended in 0.5 ml of PBS/1%FCS and finally set aside for the flow cytometry (Epics XL-MCL, Coulter).

### Results and discussion

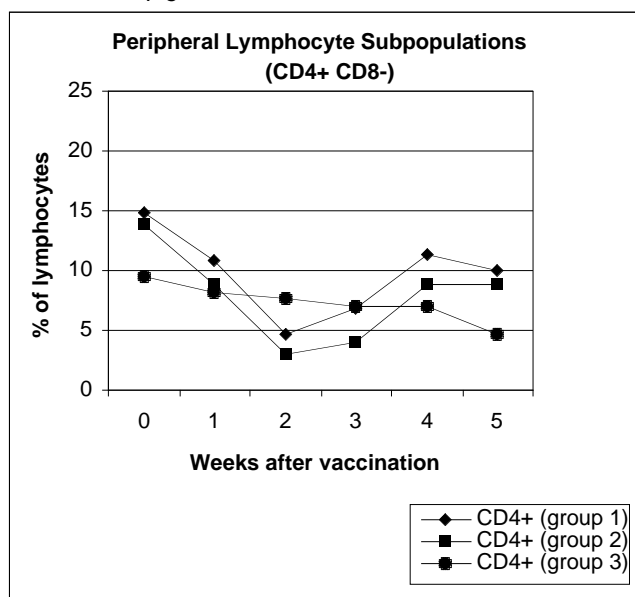
During a five weeks period after PRRS vaccination significant changes in lymphocyte subpopulations at the comparison of vaccinated and non vaccinated animals and between the two differently vaccinated groups (intramuscular and intradermal route) have been found.

One week after vaccination in both groups of vaccinated animals in comparison to control group a decrease of the percentage of CD4+CD8- cells and an increase in CD8 positive cells have been detected (Fig. 1 and 2).

The CD4+ pattern of reduction persisted at the second and at the third (only in group 2) week post-vaccination (Fig.1). The time related decrease of CD4+ cells in both vaccinated groups was significantly different compared with the control group (p<0.05). However, this change appeared transitory as at the fourth and fifth week it showed a gradual recovery of CD4+ cells values in vaccinated animals (Fig.1).

We've found that the immunomodulation caused by the vaccine mimics in several aspects the changes of peripheral T-cell subpopulations observed during experimental PRRS virus infection (8, 9).

Fig.1. Peripheral blood CD4+ cells in vaccinated and unvaccinated piglets .



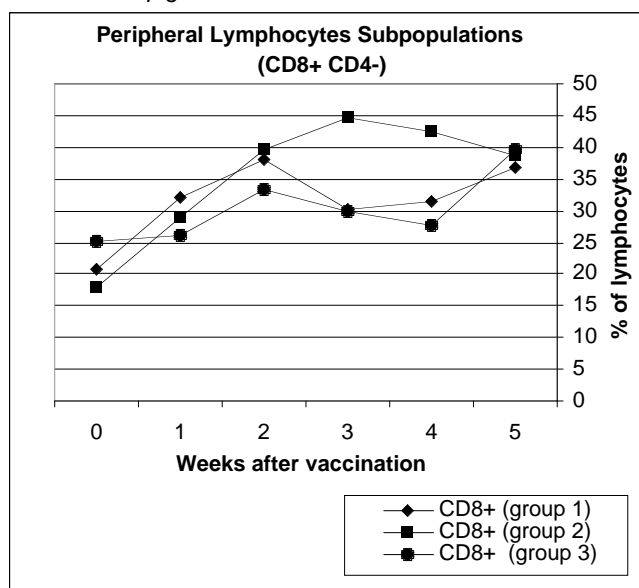
Shimizu et al (8) found a transient decrease in CD4+ cells associated with an increase of CD8+ in naturally and experimentally infected piglets (8) but they didn't explained exactly the change in CD4+ cells. At 5 weeks of age (at the start of the experiment) the immune system is surely maturing as phenotypic and functional characteristics and in this critical phase it can be particularly receptive and susceptible to antigenic stimulations. In our view, the CD4+ decrease observed in vaccinated groups (especially in group 2) could be justified as a transient chemotactic recruitment and peripheral distribution of these cells into lymphoid tissue to sustain the primary activation against the vaccine (1).

The CD8+ cells percentage increased at first and second week post-vaccination in both groups of vaccinated piglets. Thereafter, in the third week after vaccination the greatest changes and differences between vaccinated and unvaccinated animals have been observed (Fig. 2). Particularly, the CD8+ values in intradermal vaccinated animals (group 2) resulted constantly and significantly higher during the third and the fourth week post-vaccination ( $p < 0.005$ ).

The CD8+ cells increase could be referred to an earlier immune activation induced by the vaccine

However, also for the CD8+ cells, the immunomodulation induced by vaccination appears to be transient and after 5 weeks the pattern of lymphocyte subsets are similar in vaccinated and unvaccinated piglets.

Fig.2.: Peripheral blood CD8+ cells in vaccinated and unvaccinated piglets .



The increase of CD8+ T cells could be a direct consequence of the raise of lymphocytes with different phenotype and functional role which are involved in both innate and acquired cell-mediated response against viruses.

Either CD3+CD4-CD8<sup>bright</sup> (cells with cytotoxic activity MHC I restricted) or  $\gamma/\delta$  and NK cells (4,7) could be the subpopulations involved in the observed CD8+ cells increase. It's thought that an adequate stimulation of these cells is very

important for a correct activation of acquired immunity (1, 2, 4, 6). Particularly,  $\gamma/\delta$  and NK cells could play an important role as a first line of defence in PRRS infection as effectors of the innate immunity.

Cytofluorimetric studies able to differentiate cells with innate or MHC restricted cytotoxic activity could allow to better understand the efficiency of the vaccine immunomodulation activity and to evaluate the differences between the two inoculation routes (IM and ID).

Further data about the efficiency of vaccination in terms of protection and about the characteristics of the immune response will be achieved in the same piglets after a challenge infection using a heterologous virus.

## References

1. Lopez Fuertes L., Domenech N., Alvarez ., Equerra A., Dominguez J. , Castro J.m., Alonso F. (1999) Analysis of cellular immune response i pigs recovered from porcine respiratory and reproductive syndrome infection. *Virus Research* 64, 33-42.
2. Murtaugh M., Xiao Z., Zuckermann F. (2002) Immunological responses of swine to Porcine Reproductive ad respiratory Syndrome virus infection. *Viral immunology*, 15 (4), 533-47.
3. Foss D.L. Zilliox M.J., Meier W., Zuckerfmann F., Murtaugh M.P. (2002) Adjuvant danger signals increase the immune response to porcine reproductive and respiratory syndrome. *Viral immunology*, 15: 557-566.
4. Saalmuller A., Pauly T., Hohlich B.-J., Pfaff E. (1999) Characterization of porcine lymphocytes and their immune response against viral antigens. *Journal of Biotechnology* 73, 223-233.
5. Bautista E. M., Molitor T. W. (1997). Cell-mediated immunity to Porcine Reproductive and Respiratory Sindrome virus in swine. *Viral Immunology*, 10(2), 83-94.
6. Samson J. N. de Bruin T.G.M., Voermans J:J:M., Meulenber J:J:M., Pol J:M:A, Bianchi A.T.J. (2000) Changes of leukocytes phenotype and function in te broncho-alveolar lavage fluid of pigs infected with porcine reproductive and respiratory syndrome virus: a role for CD8+ cells. *J Gen Virol*, 81, 497-505.
7. Albina E., Piriou L., Hutet E., Cariolet R., L'Hospitalier R. (1998) Immune response in pigs infecetd with porcine reproductive and respiratory syndrome virus (PRRSV) *Vet Immunol Immunopathol*, 61, 49-66
8. Shimizu M., Yamada S., Kawashima K., Ohashi S., Shimizu S., Ogawa T. (1996). Changes of lymphocytes subpopulations in pigs infecetd with porcine reproductive and respiratory syndrome (PRRS) virus. *Vet Immunol Immunopathol.*, 50, 19-27.
9. Nielsen J and Botner A. (1997). Haematological and immunological parameters of 41/2 month old pigs infected with PRRS virus. *Vet Microbiol.* 55, 289-294.