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Effect of Immunosuppressive Treatment on Porcine Cytomegalovirus Infection

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Introduction

Porcine cytomegalovirus (PCMV) is one of the most prevalent infectious agents within the swine population, and there is an increased concern about PCMV due its potential to be an important infectious agent in the context of xenotransplantation. Often the disease caused by PCMV is not seen in swine herds in which the virus is endemic. The disease is more likely to be associated with recent introduction of the virus or with environmental factors such as poor nutrition and concurrent disease. Like in other cytomegaloviruses, PCMV disease is strongly associated with immunosuppression. The hypothesis tested in this experiment was that immunosuppressive therapy would render the animals more susceptible to PCMV infection, by mimicking a stress situation that would occur in the field (e.g. weaning). One of the most widely immunosuppressive drugs for human transplant recipients is cyclophosphamide. This drug is also very effective in causing immunosuppression in pigs (Mackie, 1981). The objective of this study was to establish a model of immunosuppression in pigs, using cyclophosphamide, creating conditions ideal for the establishment of PCMV infection.

Material and Methods

Eleven 3 weeks old, cesarean derived and colostrum deprived (CD-CD) pigs were used. Animals were maintained in approved animal care facilities of the University of Minnesota in accordance with the guidelines of the American Association for Laboratory Animal Care. Six pigs were treated with cyclophosphamide, every other day, at 30 mg/kg, intraperitoneally, for seven days and then they were infected with PCMV-infected cells, Japan isolate, 8 x 10^6 cells/ ml, 0.5 ml/nostril. One animal was not treated with cyclophosphamide and was infected with PCMV (no cyclophosphamide, infected control). Two animals were treated with cyclophosphamide, every other day, at 30 mg/kg, intraperitoneally, for seven days (days -7 to 0; non-infected cyclophosphamide controls), and two animals were treated with saline solution and inoculated with non-infected cells, 0.5 ml in each nostril (control animals). Clinical signs of each pig were recorded daily. Nasal and tonsil swabs and blood were collected at -7 to 0 and 0-14 days post infection (pi.). At day 14 pi., all animals were sacrificed, and tissues were collected, including brain, lung, liver, kidney, heart, lymph nodes, nasal turbinates, salivary gland and tonsils. A portion of each tissue was divided into histopathology (fixed in buffered formalin) and frozen in -70°C, for virus isolation and PCR. The major endpoints for measurement were: distribution of virus in various tissues as determined by PCR and VI and gross- and micro-pathology.

Results and conclusion

All animals treated with cyclophosphamide had a sharp decrease in the amount of white blood cells circulating in the blood (WBC count) during the seven days of treatment with cyclophosphamide. The levels of white blood cells started to increase between days 14 to 16 post treatment (days 7-9 pi.). All animals were negative by PCMV PCR at day 0. Four of the six pigs treated with cyclophosphamide and infected with PCMV had PCMV DNA in the lungs, nasal turbinates, spleen and salivary glands at the end of the experiment. The peripheral blood mononuclear cells (PBMCs) of four of the six pigs treated with cyclophosphamide and infected with PCMV had PCMV DNA from day 6 to day 14 pi. Nasal and tonsil swabs were negative in all animals through the experiment. Inclusion bodies characteristics of PCMV were observed in the nasal turbinates and lungs of four out of the six pigs treated with cyclophosphamide and infected with PCMV. No clinical signs and gross lesions were observed. Based on these results, we conclude that cyclophosphamide could cause immunosuppression in the treated pigs and that PCMV could be found in different tissues after the immunosuppressive treatment.

Table 1: PCMV-PCR results on different tissues at day 14 pi.

Tissues	1[a]	2[a]	3[b]	4[b]	5[c]	6	7	8	9	10	11
PBMCs	-	-	-	-	-	+	+	+	+	-	+
Lung	-	-	NT[d]	-	-	+	NT	+	+	-	+
Nasal turbinate	-	-	NT	-	+	+	NT	+	+	-	+
Spleen	-	-	NT	-	-	+	NT	+	+	-	+
Salivary gland	-	-	NT	-	-	+	NT	+	+	-	+

[a] Control pigs
[b] Non-infected cyclophosphamide controls
[c] No cyclophosphamide, infected control
[d] NT- non-tested (animals died before the end of the experiment)

References

Mackie, E.J. Immunosuppressive effects of cyclophosphamide in pigs. Am. J. Vet. Res., v. 42, n. 2, p.189-194, 1981.