

## **EFFECT OF BOVINE VIRAL DIARRHEA VIRUS ON MACROPHAGES FUNCTIONS**

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### **ABSTRACT**

Infection with bovine viral diarrhoea virus (BVDV) is prevalent in the cattle population worldwide. An important aspect of BVDV infection is the immunosuppression which results from virus effects on the immune cells. Macrophages were recovered from normal calves, and were exposed to BVDV *in vitro*. Tests were carried out on the recovered macrophages to determine the effects of the virus on several functional properties. CD14 receptor expression and phagocytosis were both significantly reduced in macrophages exposed to the virus *in vitro*.

### **INTRODUCTION**

Bovine viral diarrhoea (BVD) virus is a pestivirus of the family Flaviviridae. The disease is characterized by thrombocytopenia, leukopenia, fever, hemorrhages, diarrhoea and death (**Baker, 1987**). This virus is of particular interest in studies of virus-induced apoptosis because of the existence of closely related 'pairs' of cytopathic (cp) and non-cytopathic (ncp) biotypes (**Paton, 1995**). The two biotypes of such a pair differ only in one non-structural protein, NS23, which is cleaved into NS2 and NS3 in cp, but not ncp, biotypes (**Meyers & Thiel, 1996**). The macrophage is a primary key to the body's initial immune system response when faced with infection. If a macrophage becomes activated through various stimuli, an increase in metabolic rate, motility, and phagocytic activity occurs. CD14 is a multifunctional receptor constitutively expressed primarily on the surface of monocytes, macrophages, and neutrophils. It has a key role in host defense responses to microbial pathogens. CD14 has specificity for lipopolysaccharides (LPS) and other bacterial wall-derived components. An important aspect of BVDV infection is the immunosuppression which increases the host's susceptibility to secondary bacterial or viral infections. Fc receptor (FcR) and complement receptor (C3R) expression, phagocytosis and microbicidal activity and the production of neutrophil chemotactic factors were all significantly reduced in macrophages recovered from BVDV infected calves, compared with pre-inoculation control levels, whereas the control inoculated calves displayed significant increases in some of the functions. With macrophages exposed to the virus *in vitro*

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however, only FcR and C3R expression and phagocytic activity were significantly reduced (**Welsh et al., 1995**). Noncytopathic strains of bovine viral diarrhea virus prime bovine bone marrow-derived macrophages for enhanced generation of nitric oxide. BVDV, also down regulates production of tumor necrosis factor alpha in macrophages in vitro (**Adler et al., 1994 and 1996**). To continue in the efforts to evaluate the effect of BVDV infection on immunological parameters in vitro, the effect of BVDV on macrophages functions is studied.

### **MATERIALS AND METHODS**

**Separation of macrophages:** Bovine blood was collected with anticoagulant, diluted to 1:4 with Heparine-PBS and layered on Histopaque 1083 solution (Sigma). The blood was centrifuged at 1100 xg for 40 minutes. Three layers were obtained, plasma (upper), mononuclear cells (middle) and red blood cells (lower). The middle layer was gently obtained and washed one time with PBS. Total mononuclear leukocytes recovered from the interface were re-suspended in RPMI 1640 medium, 10% fetal bovine serum, and penicillin (100 µ/ml)/streptomycin (100 µg/ml). Samples (200 µL/well) of a cellular suspension ( $4 \times 10^6$  cells/ml) were then layered on 24-well plastic plates (Nunc, Roskilde, Denmark) and incubated for three hours at 37°C in an atmosphere of 5% CO<sub>2</sub>. Non-adherents cells were removed by washing with warm medium and adhered cells were used for the experiments. Monocytes differentiated after 5 days in culture into macrophages; the macrophages were evaluated by morphological criteria.

**Infection of macrophages:** Bovine macrophages were infected in vitro with a cytopathic and biotype of BVDV (NADL). Virus culture fluid was added to adhered macrophages at final concentration of  $4 \times 10^5$  (multiplicity of infection [MOI] = 0.8) and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for six hours. The control used was macrophages cultured with supplemented medium without virus.

**Direct immunofluorescence for BVDV antigens.** Macrophages were washed in phosphate-buffered saline and fixed with cold acetone for five minutes. Intracellular viral antigens were detected by a direct immunofluorescence assay using fluorescein-conjugated BVDV-specific polyclonal antibodies.

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**Effect of BVDV on macrophages receptor expression:** To determine the effect of BVDV on CD14 receptor expression, the infected macrophages were fixed with acetone and stained with commercially available specific murine monoclonal antibodies anti- CD 14 conjugated with FITC (VMRD Inc., Pullman, Washington, USA). Indirect immunofluorescence staining of macrophage was done according to the procedure of Lalor et al. 1986.

**Phagocytic activity:** The test was done to determine the effect of BVDV on phagocytic activity of macrophages according to the modified protocol of (Flipo et. al., 1992). Coated beads were diluted in phosphate-buffered saline (PBS) to an appropriate concentration. Macrophages cells in RPMI 1640 medium with no supplement were incubated at 37 °C for 45 minutes with fluorescent beads (Polyscience, Warrington, Pennsylvania, USA) at a cell/microspheres ratio of 1:100. Cells were then fixed, and the ingested beads examined with the fluorescence microscope.

## RESULTS AND DISCUSSION

Infection of bovine macrophages with BVDV resulted in the modulation of macrophage functions. As shown in figures 1, BVDV decreased CD14 cell receptor expression. The results showed variation in the percentages of cells expressing CD14, in the virus infected cells (fig.1b), when compared to the controls non infected cells (fig.1a). This negative regulation might have an impact on the host immune system because of these surface markers, are key-molecules that are involved in the interactions between immune cells in the early phase of the immune response. A fluorescent latex bead model was chosen to study phagocytosis because this model is reproducible and quantitative. The quantal increase in phagocytosis found with increasing numbers of ingested beads simplifies comparative analysis. The results showed that BVDV decreased the phagocytic function of macrophages. As shown in fig 2, there was variation in the percentages of macrophages capable of phagocytosing more fluorescent microspheres between the BVDV-infected (fig.2b) and control groups of cells (fig.2a). These results suggest that BVDV affect macrophage functions related to host defense. These observations are also consistent with the fact that BVDV has been shown to regulate the production of cytokines (Adler et al., 1996 and Perler et al., 2000) that could, in turn, have a role in the regulation of other cell

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functions such as phagocytosis. Taken together, our immunological results might indicate that BVDV isolate exerts dual effects on the host immune system as shown by the apparent down regulation in CD14 expression and the reduced cell phagocytic capability of immune cell activities.

The results agree with **Welsh et al., 1995**, who demonstrated that macrophages exposed to the virus in vitro show FcR and C3R expression and phagocytic activity were significantly reduced. However, these results are in disagreement with those reported by **Archambault et al., 2000**, where active phagocytosis was noticeable in mononuclear cells present in the bone marrow of calves exposed to the BVDV isolate. The variation between these results may be due to there experiment, where they investigated these functions in vivo. In conclusion, these results demonstrated that BVDV affected macrophages functions and suggest that BVDV infection in calves is associated with dys-regulation of certain immunological functions.

Fig.1. Effects of BVDV on macrophages cell surface expression

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Fig.1a. Control non infected macrophages

Fig. 1b. Infected macrophages: show decreased CD14 cell surface  
expression

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Fig. 2: Effect of BVDV on phagocytic activity of macrophages

Fig.2a. Control non infected macrophages

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Fig.2b. Infected macrophages: show decreased phagocytic function

#### REFERENCES

- Adler, B., Adler, H., Pfister, H., Jungi, T. W. & Peterhans, E. (1997).** Macrophages infected with cytopathic bovine viral diarrhea virus release a factor(s) capable of priming uninfected macrophages for activation-induced apoptosis. *J. Virology* **71**, 3255-3258.
- Adler H, Frech B, Meier P, Jungi TW, Peterhans E (1994).** Noncytopathic strains of bovine viral diarrhea virus prime bovine bone marrow-derived macrophages for enhanced generation of nitric oxide. *Bi.ochem Biophys Res Commun.* Aug 15;202(3):1562-8.
- Adler, H., Jungi, T. W., Pfister, H., Strasser, M., Sileghem, M. & Peterhans, E. (1996).** Cytokine regulation by virus infection: bovine viral diarrhea virus, a flavivirus, down regulates production of tumor necrosis factor alpha in macrophages in vitro. *Journal of Virology* **70**, 2650-2653.
- Archambault D, Beliveau C, Couture Y, Carman S. (2000).** Clinical response and immunomodulation following experimental challenge of calves with type 2 noncytopathogenic bovine viral diarrhea virus. *Vet Research*, 31, 215-227.
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- Baker, J. C. (1987).** Bovine viral diarrhea virus: a review. *Journal of the American Veterinary Medical Association* **190**, 1449-1458.
- Flipo D., Bernier J., Girard D., Krzystyniak K., Fournier M., (1992).** Combined effects of selected insecticides on humoral immune response in mice, *Int. J. Immunopharmacol.* **14** 747-742.
- Lalor P.A., Morrison W.I., Goddeeris B.M., Jack R.M., Black S.J. (1986).** Monoclonal antibodies identify phenotypically and functionally distinct cell types in the bovine lymphoid system, *Vet. Immunol. Immunopathol.* **13**, 121-140.
- Meyers, G. & Thiel, H.-J. (1996).** Molecular characterization of pestiviruses. *Advances in Virus Research* **47**, 53-118.
- Paton, D. J. (1995).** Pestivirus diversity. *J. of Comparative Pathology* **112**, 215-236.
- Perler L, M. Schweizer, T. W. Jungi and E. Peterhans. (2000).** Bovine viral diarrhoea virus and bovine herpesvirus-1 prime uninfected macrophages for lipopolysaccharide-triggered apoptosis by interferon-dependent and -independent pathways. *J. G. Virology*, **81**, 881-887.
- Welsh MD, Adair BM, Foster JC (1995).** Effect of BVD virus infection on alveolar macrophage functions. *Vet Immunol Immunopathol.* Jun; **46**(3-4):195-210.

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الإصابة بفيروس الإسهال البقري الفيروسي منتشرة في الماشية على مستوى العالم. من السمات المميزة لهذا الفيروس هو التثبيط المناعي الناتج عن تأثير الفيروس على الخلايا المناعية. تم تحضير خلايا المكروفاج من أبقار طبيعية وحقنها بالفيروس في المعمل. وأجريت الاختبارات على هذه الخلايا لمعرفة تأثير الفيروس على القدرة الوظيفية لها. حدث تثبيط لإنتاج مستقبلات الخلية س د ١٤ وكذلك قدرة الخلية على التهام الأشياء.

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