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# The Role of B Cells in the Immune Response to Pestivirus (Classical Swine Fever Virus)

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## Summary

Pigs inoculated with the Alfort 187 isolate of classical swine fever (CSF) virus were used to study the immunological mechanisms associated with the humoral immune response in the disease. Quantitative and qualitative changes in the B-cell population (lambda light chain [C-λ]-positive, immunoglobulins [Ig]-M-positive, and IgG-positive were demonstrated in the spleen, thymus and ileocaecal lymph node. Blood and serum samples were used to examine changes in leucocytes, albumin/globulin ratios and specific antibodies against CSF virus titration. Despite the lymphoid depletion shown by infected animals, an increase in B cells and potentially immunoglobulin-producing C-$\lambda^+$ plasma cells was observed in the lymphoid organs from the onset of disease. The increase in C-$\lambda^+$ B cells was matched by a parallel increase in $IgM^+$ cells, which attained peak values from 7 days post-inoculation (dpi), while $IgG^+$ cells increased from 11 dpi onwards. The enhanced biosynthetic capacity of these cells may have been linked to the initiation of a humoral response to CSF virus, and to the progressive decline in the albumin/globulin ratios of inoculated animals. Activation, proliferation and differentiation of B cells coincided with the presence of viral antigen, and with an intense phagocytic and biosynthetic activity of monocytes-macrophages and T lymphocytes. The previously reported increase of cytokine (TNFα, IL-1α and IL-6) production by monocytes-macrophages, and the release of IL-2, IL-4 and IFNγ by T lymphocytes, may play a role in the initiation of the humoral immune response in CSF. These changes may have influenced the late appearance of virus-specific antibodies in the study, as well as the progressive increase of immunoglobulins.



*Keywords:* classical swine fever; immune response; lymphoid depletion; pig; swine fever; viral infection

## Introduction

Initiation of the immune response depends on antigen-presenting cells and T cells, whose activation and proliferation are regulated by specific antigen–receptor interactions; these interactions in turn depend on cell adhesion molecules and cytokines (Brodsky and Guagliardi, 1991). Activated T cells provide stimulatory signals enabling B cells to proliferate and subsequently differentiate into immunoglobulin-producing plasma cells; several cell surface molecules and their cytoplasmic domains also participate in this interaction (Parker, 1993; Clark and Ledbetter, 1994). Few studies have investigated the mechanisms of this interaction in classical swine fever (CSF), a disease characterized by initial leucocytosis followed by severe leucopenia (Trautwein, 1988; Susa *et al.*, 1992; Sato *et al.*, 2000) affecting both peripheral-blood B lymphocytes (Lee *et al.*, 1999) and T lymphocytes (Pauly *et al.*, 1998; Summerfield *et al.*, 1998; Markowska-Daniel *et al.*, 1999); little is known of the role played in the humoral immune response by the cell populations remaining after lymphocyte destruction.

According to recent studies on CSF, macrophage populations at a number of sites display intense phagocytic and biosynthetic activity, with increased production of cytokines including tumour necrosis factor (TNF)α, interleukin (IL)-1α and IL-6 (Sánchez-Cordón *et al.*,

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2002, 2005b; Núñez *et al.*, 2005). These changes coincide with an increase in T-lymphocyte numbers and in cytokine expression (interferon [INF-$\gamma$], IL-2 and IL-4) (Sánchez-Cordón *et al.*, 2005a), as well as with the presence of viral antigen. However, the role played by these events in the induction of the humoral immune response in CSF remains far from clear. Study of the antigen-prompted production of certain chemical mediators may help to evaluate the immune response (Wood and Seow, 1996; Mateu de Antonio *et al.*, 1998); thus, the presence of such mediators may be recorded in conjunction with changes in B- and T-lymphocyte populations and with cytokine and immunoglobulin expression by these lymphocytes.

Full knowledge of the humoral immune response to CSF virus is essential to a better understanding of host defence mechanisms. Some in-vitro studies have identified neutralizing antibodies against CSF virus, which appear capable of controlling viral replication (Terpstra and Wensvoort, 1988); other authors report non-detectable concentrations of neutralizing antibodies but suggest that T cells play a protective role (Rümenapf *et al.*, 1991; Pauly *et al.*, 1995). Moreover, research on the immune response to CSF virus is often restricted to specific antibodies or other serum proteins (Islas *et al.*, 1997; Laevens *et al.*, 1999; Piriou *et al.*, 2003); only a few authors have examined tissue samples to investigate the possible role of B cells (Narita *et al.*, 1996, 2000) in the pathogenesis of the disease.

The aim of this work was to study the immunological mechanisms associated with the humoral immune response of pigs to inoculation with the Alfort 187 strain of CSF. Spleen, thymus and ileocaecal lymph nodes are highly appropriate for studying the pathogenesis of CSF because, despite the difficulties in identifying lymphocyte populations in paraffin wax-embedded tissues, they show significant histopathological changes and diverse immunocompetent cell populations (Fox *et al.*, 1985; González *et al.*, 2001). Evaluation of the immune response in CSF requires a quantitative and qualitative study of B cells in the lymphoid organs, in parallel with an investigation of changes in blood leucocytes, albumin/globulin ratios and virus-neutralizing antibodies.

## Materials and Methods

### *Animals, Virus and Experimental Design*

Experimental design was previously reported by Sánchez-Cordón *et al.* (2002). Briefly, 40 healthy Large White × Landrace pigs of either sex, aged 4 months, were used. Thirty-six animals were each inoculated intramuscularly with $10^5$ $TCID_{50}$ of the virulent CSF isolate "Alfort 187" (Wensvoort *et al.*, 1989). Four animals, used as controls, received only phosphate-buffered saline (PBS), pH 7·2.

Thirty-two pigs were sedated and killed in batches of four at 2, 3, 4, 7, 9, 11, 14 and 15 days dpi. Four control pigs were killed at 15 dpi, together with the final batch of infected animals (nos 376, 378, 380 and 389).

### *Blood Collection and Specific Antibody Detection in Serum*

Pre-inoculation blood samples were taken from the jugular vein of all pigs to obtain baseline values. A further blood sample was taken from each infected pig immediately before slaughter. Blood tubes containing EDTA 1% were used for blood leucocyte counts with a haemocytometer, while tubes without anticoagulant were used to obtain serum, which was then frozen at −80 °C until tested. Total albumin and total globulin in serum were measured by a densitometer (BTS–235; Biosystems, Barcelona, Spain).

In addition, blood samples from the four inoculated pigs killed at the end of the experiment (15 dpi), were taken at 1, 2, 3, 4, 5, 6, 7, 9, 11, 14 and 15 dpi. Specific antibody titres in the serum of animals from this group were determined by the CEDITEST® (Instituut voor Dierhouderij en Diergezondheid, Lelystad, The Netherlands).

### *Histopathology and Immunohistochemistry (IHC)*

Samples of spleen, thymus and ileocaecal lymph nodes were fixed in 10% buffered formalin solution 0.01 M, pH 7·2 for 24 h or Bouin's solution for 8 h. Samples were then processed by routine methods and embedded in paraffin wax.

For histopathological examination, sections (4 μm) of formalin-fixed tissue were cut and stained with haematoxylin and eosin. For IHC, the avidin–biotin–peroxidase complex (ABC) method (Hsu *et al.*, 1981), was used. Details of the primary antibodies, including dilutions and pre-treatments, are summarized in Table 1. CSF viral glycoprotein E2 (gp55) was demonstrated with the monoclonal antibody WH303 in sections (3 μm) of Bouin's solution-fixed samples. The identification of B-lymphocyte populations and cells expressing immunoglobulins was carried out on sections of formalin-fixed tissue samples. Tissue sections in which the specific primary antibodies were replaced by rabbit or mouse non-immune serum, constituted negative controls. Samples from the four uninoculated animals were also used as controls.

### *Statistical Analysis*

To calculate the number of immunolabelled cells present and to relate the results obtained with the different antibodies, two paraffin-wax blocks from