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Evolution of T Lymphocytes and Cytokine Expression in Classical Swine Fever (CSF) Virus Infection

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Summary

This study characterized the cell-mediated immune response in pigs inoculated with the Alfort 187 isolate of classical swine fever (CSF) virus. Quantitative changes in the T-lymphocyte population ($CD3^+$, $CD4^+$ and $CD8^+$) and qualitative changes in cytokine expression (IL-2, IL-4 and IFNγ) by these cells in serum, thymus and spleen were demonstrated. These changes coincided spatially and temporally with previously described quantitative and qualitative changes in monocyte-macrophage populations, thus demonstrating the contribution of the two cell populations to lymphoid depletion. Moreover, examination of cytokine expression in thymus and spleen samples revealed a type 1 cell-mediated immune response in the early and middle stages of the experiment, giving way to a type 2 immune response towards the end of the experiment; these findings, which accorded with the serological results and lymphopenia, may influence the delayed humoral response characteristic of CSF.

Keywords: cell-mediated immunity; classical swine fever; cytokine expression; lymphoid depletion; pig; spleen; swine fever; thymus; viral infection

Introduction

Macrophages, dendritic cells and natural killer cells play an important role in immunity against infectious diseases. The functions of these cells include the uptake and killing of intracellular pathogens, lysis of infected host cells, presentation of antigens to T cells and release of cytokines or chemical mediators that activate macrophages or direct the induction of T-cell subtypes. These cytokines and chemokines recruit leucocytes to the site of infection or injury, activate their antimicrobial function and regulate the induction of the adaptative response to the pathogen (McGuirk and Mills, 2000; Ryan *et al.*, 2000).

When the innate immune response fails to control the infection, the adaptative immune response is activated and this involves the production of antibodies and primed T cells. $CD8^+$ cytotoxic T lymphocytes (CTLs) kill target cells infected with viruses or bacteria, whereas $CD4^+$ T-helper (Th) cells provide help for B cells in antibody production and secrete cytokines that play a role in immunoregulatory functions or have a direct effect on invading pathogens (Germain, 1994; Jondal *et al.*, 1996; Tizard, 1998). Thus, interferon (IFN)γ and interleukin (IL)-2 are secreted during the so-called type 1 immune response, while IL-4, IL-5, IL-6, IL-10 and IL-13 are present in the type 2 immune response (Karupiah, 1998; Hernández *et al.*, 2001; McGuirk and Mills, 2002).

The functions of IFNγ include the stimulation of immunoglobulin production (Boehm *et al.*, 1997; Samuel, 2001) and specific cytotoxicity of T cells (Bach *et al.*, 1997; Biron and Sen, 2001; MacDonald

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et al., 2002), induction of apoptosis (Tanaka *et al.*, 1998; Samuel, 2001) and activation of resting tissue macrophages (Bach *et al.*, 1997; Biron and Sen, 2001), thereby enhancing resistance against viral infections (Mateu de Antonio *et al.*, 1998; Samuel, 2001). IL-2 induces growth and proliferation of T cells (Stasny *et al.*, 2001) and stimulates their cytotoxicity (Murtaugh, 1994; Mosmann and Sad, 1996; MacDonald *et al.*, 2002), supporting the proliferation of B cells. IL-4 promotes the development of helper and cytotoxic T cells and the differentiation of immunoglobulins-producing plasma cells from B cells (Murtaugh, 1994; Van Miert, 1995; Mosmann and Sad, 1996).

The monocyte-macrophage (m-M∅), identified as the main target cell for classical swine fever (CSF) virus (Summerfield *et al.*, 1998; Gómez-Villamandos *et al.*, 2001; Sánchez-Cordón *et al.*, 2003), exhibited phagocytic and secretory activation leading to the synthesis and release of various chemical mediators (tumour necrosis factor [TNF]α, IL-1α and IL-6) associated with the modulation of the inflammatory and immune responses, and with the appearance of lesions characteristic of the disease (Gómez-Villamandos *et al.*, 2000; Bautista *et al.*, 2002; Sánchez-Cordón *et al.*, 2002, 2003). The cellular immune response, the characteristics of T lymphocytes and their activity against CSF virus have been studied mainly by in-vitro methods, with leucocytes isolated from peripheral blood (Pauly *et al.*, 1995; Summerfield *et al.*, 1996, 2001); only a few such studies have been carried out on tissue samples (Narita *et al.*, 1996, 2000; Pauly *et al.*, 1998).

Despite the difficulty of identifying T-lymphocyte subtypes in paraffin wax-embedded, fixed tissues (Fox *et al.*, 1985; González *et al.*, 2001), such material may be useful in studying the interaction between different cells of the immune system, the presence of antigen, and the lesions. Moreover, information on immunity may be gained by demonstrating increased levels of chemical mediators, as they are directly related to the T-lymphocyte response induced by antigen (Wood and Seow, 1996; Mateu de Antonio *et al.*, 1998).

Elucidation of the mechanisms responsible for evasion of the immune response by CSF virus should lead to a better understanding of the pathogenesis of human and animal diseases caused by flaviviruses (Burke and Monath, 2001).

The purpose of this CSF study was to explore the distribution and evolution of T-lymphocyte populations and the cytokines released by them in thymus and spleen, and to determine their role in pathogenesis.

Materials and Methods

Animals, Virus and Experimental Design

Large White x Landrace pigs ($n=36$) of either sex, aged 4 months and weighing *c.* 30 kg were used; they were serologically negative for CSF, African swine fever, porcine reproductive and respiratory syndrome and Aujeszky's disease. All animals were housed in the Centro de Investigación en Sanidad Animal in Valdeolmos, Madrid, Spain. Thirty-two pigs each received an intramuscular inoculation of 10^5 50% tissue culture infective doses ($TCID_{50}$) of the virulent CSF virus isolate "Alfort 187" (Wensvoort *et al.*, 1989). The remaining four animals (controls) received only phosphate-buffered saline (PBS), pH 7.2. Clinical signs and rectal temperature were then monitored daily. The experiment was carried out in accordance with the Code of Practice for Housing and Care of Animals used in Scientific Procedures, approved by the European Economic Community Union in 1986 (86/609/EEC).

Blood Collection and Enzyme-linked Immunosorbent Assay (ELISA)

Preinoculation blood samples were taken from all pigs to obtain baseline values. Blood samples were taken aseptically from the anterior vena cava at 2, 3, 4, 5, 6, 7, 9, 11, 14 and 15 days post-inoculation (dpi) for leucocyte counts by means of a haemocytometer and to obtain sera for ELISA. Commercial ELISA kits were used to measure IFNγ (Swine IFNγ Cytoscreen; Biosource, Camarillo, CA, USA), IL-2 (Swine IL-2 CytoSets; Biosource) and IL-4 (Swine IL-4 Cytoscreen; Biosource), according to the manufacturer's instructions. Absorbency of ELISA plates was measured by spectrophotometry (Easy Reader EAR 400; SLT-LabInstruments, Salzburg, Austria).

Processing of Specimens for Structural Study and Immunohistochemistry (IHC)

Infected pigs were sedated with azaperone (Stresnil®; Jannsen Animal Health, Beerese, Belgium) and killed by a overdose of thiopental-sodium (Thiovet®; Vet Limited, Leyland, Lancashire, England) in batches of four at 2, 3, 4, 7, 9, 11, 14 and 15 dpi; The remaining four pigs (controls) were killed at the end of the experiment. Samples of thymus and spleen were fixed in 10% buffered formalin solution (pH 7.2) and Bouin's solution for structural and immunohistochemical study.