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Expression of Interferon-α and Mx Protein in the Livers of Pigs Experimentally Infected with Swine Hepatitis E Virus

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Summary

Expression of interferon-α (IFN-α) and Mx protein in the livers of pigs experimentally infected with swine hepatitis E virus (HEV) was examined immunohistochemically. Five infected and five non-infected pigs were killed at 3, 7, 14, 21, 29 and 50 days post-inoculation (dpi). Tissues were collected from each pig at necropsy. Multifocal lymphoplasmacytic hepatitis ($P = 0.042$) and focal hepatocellular necrosis ($P = 0.037$) were significantly more frequent in the swine HEV-infected pigs than in the non-infected control pigs. Immunohistochemical analysis detected expression of IFN-α and Mx protein by macrophages/Kupffer cells, lymphocytes and hepatocytes in the livers of infected pigs.

Keywords: hepatitis E virus; immunohistochemistry; interferon-α; Mx protein; pig

Introduction

Hepatitis E virus (HEV) is a non-enveloped, single-stranded, positive sense RNA virus and the sole member of the genus *Hepevirus* in the family of Hepeviridae (Emerson *et al.*, 2004). Isolates of HEV can be divided into four genotypes, principally characterized by geographical distribution, host range and pattern of infection (Mushahwar, 2008). HEV is recognized as a major cause of enterically transmitted non-A, non-B hepatitis, which is no longer confined to developing countries (Meng *et al.*, 1997; Panda *et al.*, 2007). Swine HEV, closely related to human HEV, was discovered in swine in the USA and was designated as swine HEV in 1997 (Meng *et al.*, 1997). Microscopically, naturally and experimentally HEV-infected pigs show evidence of hepatitis characterized by mild to moderate multifocal and periportal lymphoplasmacytic hepatitis, with mild focal hepatocellular necrosis (Meng *et al.*, 1997; Halbur *et al.*, 2001).

The innate immune responses that are initiated immediately after exposure to a viral pathogen are critical for the control of viral replication and virus dissemination (Haller *et al.*, 2007a, b). The interferon-induced Mx protein is one of the best studied determinants of innate immunity to viral infection and is accepted as a sensitive marker of interferon (IFN) activity (Haller et al., 2007a, b). MxA was shown to be expressed in hepatocytes of human patients naturally infected with hepatitis C virus (HCV), a single-stranded, positive sense RNA virus like HEV (MacQuillan *et al.*, 2002). The up-regulation of MxA protein seen in patients chronically infected with HCV reflects activation of the endogenous IFN-α pathway (MacQuillan *et al.*, 2002). In addition, a beneficial effect of IFN-α has been reported in patients chronically infected with hepatitis B or C virus (Hoofnagle, 1997; Stertz *et al.*, 2007). Strains of HEV from a patient with acute HEV infection were found to have 92–97% nucleotide sequence identity with a strain of HEV of swine origin (Meng *et al.*, 1998; Pina *et al.*, 2000). Thus, it would be useful to determine the expression patterns

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of IFN-α and MxA protein in pigs infected with swine HEV for potential application to the prevention and treatment of human patients with HEV infection. However, it is not known whether IFN-α and MxA proteins are expressed in pigs infected with swine HEV. The aim of the present study was to investigate by immunohistochemistry (IHC) the expression patterns of IFN-α and Mx protein in the livers of pigs experimentally infected with swine HEV.

Materials and Methods

Experimental Design

The swine HEV used in this study was isolated from pigs in Kyounggi Province (Choi and Chae, 2003). Sixty pigs, 3 weeks of age, were purchased from a commercial vendor and randomly allocated to an infected or a control group, having been confirmed as seronegative for HEV infection by an enzyme-linked immunosorbent assay (ELISA; Genelabs Diagnostics, Singapore), for porcine circovirus 2 (PCV2) by an indirect immunofluorescence test and for porcine reproductive and respiratory syndrome virus (PRRSV) by ELISA (IDEXX Laboratories, Westbrook, ME). After group allocation, the pigs were maintained in stainless steel isolators (two pigs per isolator).

An infectious pool of swine HEV was prepared as a 10% suspension of faeces collected from a colostrum-deprived pig experimentally infected with swine HEV (Meng *et al.*, 1998). The faeces of the colostrum-deprived pig were negative for several bacterial pathogens, including *Lawsonia intracellularis*, *Brachyspira hyodysenteriae*, *Brachyspira pilosicoli*, *Salmonella typhimurium* and pathogenic *Escherichia coli*, and several viral pathogens including PCV2, PRRSV, classical swine fever virus, porcine epidemic diarrhoea virus and transmissible gastroenteritis by polymerase chain reaction (data not shown). Each of 30 pigs assigned to the infected group was then inoculated intravenously with 5 ml of $10^{4.5}$ 50% pig infectious doses per ml of inoculum, as previously described (Meng *et al.*, 1998). The 30 pigs in the control group were sham inoculated with normal phosphate buffered saline (PBS; pH 7.4, 0.001 M). Five pigs from each group were killed at 3, 7, 14, 21, 29 and 50 days post-inoculation (dpi). Tissues were collected from each pig at the time of necropsy examination. All of the methods used were previously approved by the Seoul National University, Institutional Animal Care and Use Committee.

In-situ Hybridization

In-situ hybridization for swine HEV was performed as previously described (Choi and Chae, 2003).

Immunohistochemistry

Two primary antibodies were applied: (1) monoclonal mouse anti-human MxA protein (Flohr *et al.*, 1999), which reacts with porcine Mx protein (personal communication, Dr. Haller) and was kindly provided by Dr. O. Haller from the University of Freiburg, and (2) monoclonal mouse anti-pig IFN-α (Pierce Biotechnology Inc., Meridian, IL; L'Haridon *et al.*, 1991). Monoclonal mouse anti-human Mx protein antibody was diluted 1 in 100 and monoclonal mouse anti-pig IFN-α antibody was diluted 1 in 50 in PBS containing 0.1% Tween 20. Immunohistochemical analysis for MxA protein and IFN-α was performed as previously described (Jung and Chae, 2006). Lung tissue from a pig experimentally infected with PRRSV was used as a positive control for immunohistochemical analysis of MxA (Chung *et al.*, 2004).

Morphometric Analysis

Immunohistochemical labelling of Mx and IFN-α antigen was assessed in 10 randomly selected high-power fields (×400) and given a ranked score of 0–4 using a semi-quantitative system: 0, no immunohistochemical signal; 1, <1% cells labelled; 2, 10–30% cells labelled; 3, 10–30% cells labelled; and 4, >30% cells labelled.

Statistical Analysis

The Wilcoxon matched rank sum test was used to compare immunohistochemistry scores for IFN-α and Mx protein at each time point with the scores from the previous time point. The immunohistochemistry scores were analyzed to detect any relationships between expression of IFN-α protein, Mx protein and swine HEV by the Pearson correlation analysis. $P < 0.05$ indicated statistical significance.

The presence or absence of hepatic lesions was analyzed by Fisher's exact test. Lymphoplasmacytic hepatitis lesion scores were analyzed by logistic regression. The lesion score was the dependent variable and was considered an ordinal variable for the analysis. Explanatory variables included infection group (nominal variable) and the dpi (ordinal variable). Liver sections were given lesion scores with respect to the estimated quantity of lymphoplasmacytic hepatic lesions, as previously described (Halbur *et al.*, 2001). The scores ranged from 0 (no inflammation) to 4 (>10 focal infiltrates/10 hepatic lobules). The lymphoplasmacytic hepatitis lesion scores were analyzed to determine whether there was a relationship with expression of IFN-α protein and Mx protein by Pearson correlation analysis. The Wilcoxon matched rank sum test was used to compare the hepatic lesion