Identification and Sequence Analysis of the Glycoprotein B Gene of Porcine Cytomegalovirus

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Abstract. Porcine cytomegalovirus (PCMV) is one of the pathogens that should be eliminated from pigs intended for use as organ donors in xenotransplantation. For this purpose, reliable diagnostic test systems are needed. To provide a basis for this goal and to analyse the evolutionary relationships of PCMV within the herpesvirus family, the putative glycoprotein B (gB) gene of PCMV was identified by assuming gene colinearity and a relative conservation of nucleotide sequences in comparison with closely related herpesviruses. Using this approach the complete nucleotide sequence of the PCMV gB gene was determined. A protein of 860 amino acids was deduced and a putative cleavage site, conserved cysteine residues, as well as potential N-terminal glycosylation motifs were identified. In a comparison of PCMV gB with the corresponding region of other herpesviruses, the highest identities were found with human herpesviruses 6 and 7 (HHV-6 and 7; 43.4% and 42.6%, respectively). Also in phylogenetic analysis, the PCMV gB clustered with HHV-6 and HHV-7. Between the complete gB sequences of five different PCMV strains and isolates from the United Kingdom, Germany, Spain, Japan and Sweden, differences of 3.4% were found, indicating a considerable intra-species variation. The characterisation of the protein deduced from the identified gene provides further evidence that this is indeed the gB gene of PCMV and provides important taxonomical information regarding PCMV. The identification of the gB gene should facilitate the development of sensitive and robust diagnostic methods for the PCMV screening of pigs.

Key words: PCMV, porcine cytomegalovirus, herpesviridae, Betaherpesvirinae, xenotransplantation, glycoprotein B

Introduction

The pig is currently being considered as a suitable donor of organs and tissues to humans. However, the potential risks of xenogenic infections need to be addressed. The risk is increased by the immunosuppressive treatment of transplant recipients and because transplantation may help infectious agents to cross-infection barriers. It is therefore essential to establish herds of donor pigs free from known virus infections. Herpesviruses are a major concern because of the difficulties of eliminating them, caused largely by their establishment of latent infections and, in many cases, their ability to cross the placental barrier and infect foetuses. The pig may be infected with a number of herpesviruses such as porcine lymphotrophic herpesviruses 1 and 2 (PLHV-1 and PLHV-2, see Ref. [1]) and porcine cytomegalovirus (PCMV, SuHV-2). PCMV has been classified as a member of the Betaherpesvirinae [2,3]. This virus has a relatively close relationship to certain human herpesviruses and may fall into a distinct genus within the Betaherpesvirinae together with human herpesviruses 6 and 7 (HHV-6
and 7) [4,5]. A previous work [4] has indicated a relatively high genetic variation between PCMV strains and isolates. PCMV causes latent or persistent infections, is excreted intermittently and can cross the placental barrier to cause in utero infections, in spite of circulating antibodies [6–9]. The virus has a worldwide distribution and a very high prevalence at the herd level [6,10]. Creating pig herds that are free from PCMV may therefore prove difficult. To achieve and verify this and to perform post-transplantation screening of transplant recipients, sensitive diagnostic tools are needed. For the detection of PCMV DNA various PCR tests have been developed [4,5]. Detection of antibodies and antigen currently rely on immunofluorescence methods based on replication of PCMV in pig alveolar macrophages [6,10,11]. ELISA tests for detection of anti-PCMV antibodies have also been developed [12,13], but these have not been widely adopted. Production of antigen for ELISA is difficult because good in vitro cultivation systems are lacking. PCMV replicates slowly and non-lytically in porcine alveolar macrophages (PAM) and porcine fallopian tube (PFT) cells [6,11]. However, titers in PAMs are generally low, usually around log 2.0–2.5 TCID/50 (M. Faithfull, personal communication) while the use of PFT has proven unsatisfactory in our hands. The low titers and poor reproducibility of in vitro cultivation systems impairs the suitability of such assays for use in diagnosis and for production of ELISA antigen. The latter problem could be overcome by using PCMV proteins expressed by a recombinant vector system. Since the glycoprotein B (gB) is an essential protein for herpesvirus infection and generally a highly potent immunogen [14], this protein is a possible candidate for ELISA development.

The purpose of the present investigation was to identify and characterize the gB gene of PCMV in order to provide more information regarding the taxonomical position of this virus and to study evolutionary aspects of the betaherpesvirus subfamily. The studies were also focused on the potential use of the encoded protein in serological assays to detect PCMV infection.

Methods

Samples

German lung sample #489 was collected at the abattoir in Eberswalde, Brandenburg, Germany. The British strain B6 was provided as supernatant from PCMV infected porcine alveolar macrophages by Prof. N. Edington, Royal Veterinary College, London, UK. The Spanish lung sample #55 was provided by Dr. M. Domingo, University of Barcelona, Spain. The Japanese strain OF-1 was provided as supernatant by Prof. Kawamura, University of Osaka, Japan, from a PCMV infected PFT cell line. The Swedish spleen sample #1469 was provided by the pathology department, NVI, Uppsala, Sweden. The British spleen sample #P1 was provided by Dr. S. Done, Veterinary Laboratories Agency, Weybridge, UK.

DNA Preparation

DNA from tissue samples was extracted with the Qiagen DNeasy tissue kit according to the manufacturer's instructions. Supernatants from tissue cultures were boiled for 5 min. The extracted DNA was stored at −20 °C until use.

Genome Walking

Starting from the 5’-end of the DNA polymerase sequence of PCMV (accession number AF268042), upstream sequences were amplified by genome walking. This was done as described previously [15] by Genexpress GmbH, Berlin, through amplification from adaptor-ligated restriction fragment libraries using a nested-PCR approach [16]. From the resulting sequences, PCMV-specific PCR primers were designed. These were used for PCR amplification and sequencing of the remaining gaps and to repeat the sequencing of regions determined by genome walking as well as to amplify and sequence the homologous regions from the samples mentioned above.

Specific PCR

Specific PCR amplification was performed as follows: Amplitaq Gold (Roche Molecular Systems Inc, Branchburg, USA) was activated by heating to 95 °C for 12 min followed by 42 cycles with 60 s at 95 °C, 60 s at 50–60 °C (see Table 1), 2 min at 72 °C and a final extension step for 10 min at 72 °C. PCR was performed in 25 μl volumes in a MJ research PTC 200 thermocycler or in a Perkin Elmer 2400 Gene Amp thermocycler.