Analysis of HCMV glycoprotein B DNA fragments with non-radioactive and radioactive SSCP

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Human Cytomegalovirus (HCMV) is a member of the Herpesvirus family. Reactivation of latent infection has increasingly become a factor contributing to the morbidity and mortality associated with AIDS and organ transplantation. Viral transmission by the donor organ is most likely in patients HCMV seronegative prior to transplantation receiving a graft from a seropositive donor[4]. These patients are at high risk of acquiring primary infection. Latent cytomegalovirus can reactivate in seropositive patients after organ transplantation or infection with HCMV of a donor strain. The major immunogenic envelope glycoprotein B (gB) of HCMV is expressed as a precursor molecule of about 906 amino acids that is cleaved at a specific proteolytic site (Aa 460/461) to form a disulphide-linked complex. The major variable locus spans the gB proteolytic cleavage site region from codon 448 to 480 [1,2,5].

Mutations in amplified DNA fragments can be detected by single-strand conformation polymorphism analysis (SSCP). This method provides a rapid and sensitive method for identifying known and unknown mutations and polymorphisms in amplified DNA fragments. SSCP is most frequently performed using radioactive labelled PCR products[3]. Several non-radioactive detection procedures with silver staining or ethidium bromide staining are employed. We also used radioactive SSCP analysis and developed another non-radioactive SSCP detection method, which is based on the use of biotinylated primers. After electrophoresis and membrane transfer, biotinylated DNA can be detected with an enzymatic dye reaction (streptavidin alkaline phosphatase conjugate) or chemiluminescence.

For sample preparation, a simple alkaline lysis procedure of buffy coat leukocytes was performed[4]. As primers sense HCMVgB-1 (nt 1276–1295) and antisense HCMVgB-2 (nt 1505–1524), which covered all known HCMV glycoprotein B sequences, were used[1,2,5]. After PCR under standard conditions 5 µl of nonradioactive PCR product (2 µl of radioactive product) were diluted with 5 µl (8 µl) formamide dye. Double stranded DNA fragments were denatured by heating for 5 min to 95 °C and were incubated for 5 min in icewater. Five µl of the diluted solutions were transferred to a 0.5 × MDE gel (AT Biochem, USA) in a standard vertical sequencing apparatus. Gels were run with constant power (6.5 Watt) for 16 h. Separated PCR products were transferred to positively charged nylon membranes by dry blotting, crosslinked using UV light and detected as recommended by the supplier (Boehringer Mannheim, FRG), but streptavidin-AP was used instead of anti-DIG-AP antibodies. The radioactive ssDNA was detected by autoradiography[3].

The combination of biotinylated sense and non-biotinylated antisense primers allows the identification of sense and antisense strands. Because the genetic information is contained in both DNA strands, the use of only one biotinylated primer allows detection of mobility shifts (mutations). This facilitates the analysis of SSCP gels, especially when in the case of virus variability analysis of more than one virus strain in the sample is necessary.

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Detection of cytotoxin gene (vacA) and cytotoxin-associated antigen (cagA) of *Helicobacter pylori* by using the polymerase chain reaction (PCR)

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In 1987 Leunk et al.[1] described a vacuolating cytotoxic effect of *H. pylori* on cell cultures. This cloned cytotoxin[2] is an immunogenic protein with a molecular weight of 87 kDa. Its expression is linked to a surface exposed immunodominant antigen with a molecular weight of 127 kDa. This cagA antigen is used in serological tests and its gene has been cloned and sequenced[3].

Telford et al.[4] demonstrated the cytopathic effect of the cytotoxin in combination with the cagA antigen in a mouse model and concluded that the cytotoxin gene is present but not expressed in noncytotoxic strains, whereas the cagA gene is completely absent in cytotoxin negative strains.